



SECOND EDITION

FRANKHAM  
BALLOU  
BRISCOE

# INTRODUCTION TO CONSERVATION GENETICS

CAMBRIDGE

# Introduction to Conservation Genetics, Second edition

This established author team brings the wealth of advances in conservation genetics into the new edition of this introductory text, including new chapters on Population Genomics and Genetic Issues in Introduced and Invasive Species. They continue the focussed learning features for students – main points in the margin, chapter summaries, strong support with the math, and further reading – and now guide the reader to software and databases. Many new references reflect the expansion of this field. With examples from mammals, birds, reptiles, fish, amphibians, plants and invertebrates, this is an ideal introduction to conservation genetics for a broad audience. The text tackles the quantitative aspects of conservation genetics, and has a host of features to support students learning the numerical side of the subject. Combined with being up to date, its user-friendly writing style and an elegant illustration program make this a robust teaching package.

Emeritus Professor DICK FRANKHAM holds honorary appointments at Macquarie University, James Cook University and The Australian Museum and was Hrdy Visiting Professor at Harvard University in 2004. He began his career in quantitative genetics, achieving international recognition for his work on *Drosophila* before turning to conservation genetics in the early 1990s. He has made many significant contributions to the field via modelling problems in *Drosophila*, meta-analyses and computer simulations. He is a major figure in the discipline and was awarded a D.Sc. by Macquarie University in 2006 for his scientific contributions to conservation and evolutionary genetics.

Dr JON BALLOU is Population Manager and Research Scientist at the Smithsonian Institution's National Zoological Park in Washington, DC, USA and for 2003–2006 was Head of its Department of Conservation Biology. He is also an adjunct member of the Faculty of the University of Maryland. His research has focused on the genetic and demographic problems confronted by

small populations, especially of threatened species. He is recognized as a leader in developing the theoretical basis for the genetic management of small populations and in developing population management tools (software, applied theory) that are widely and internationally used by wildlife and zoo managers.

Professor DAVID BRISCOE retired in 2009 after 34 years at the Department of Biological Sciences, Macquarie University, Sydney, Australia where he was Head of Department 2006–2009. He collaborated with Dick Frankham on *Drosophila* research for 34 years, as well as working on the evolutionary genetics of rock wallabies, lizards, velvet worms, social insects and slime moulds. An outstanding communicator, his inspirational teaching enthuses students at all levels and reaches beyond the academic sphere through television appearances and popular books.

# Introduction to Conservation Genetics

Second Edition

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# Contents

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*Preface to the second edition*

*Preface*

*Copyright acknowledgments*

## Chapter 1 Introduction

The 'sixth extinction'

Why conserve biodiversity?

Endangered and extinct species

What is an endangered species?

What causes extinctions?

Recognition of genetic factors in conservation biology

What is conservation genetics?

Examples of the use of genetics to aid conservation

Genetic management of threatened species

Methodology in conservation genetics

Sources of information

Summary

General bibliography

Problems

Practical exercises: categorizing endangerment of species

## Chapter 2 Genetics and extinction

Genetics and the fate of endangered species

Relationship between inbreeding and extinction

Relationship between loss of genetic diversity and extinction

Summary

Further reading

Software

Problems

Practical exercises: computer projections

## **Section I Evolutionary genetics of natural populations**

## Chapter 3 Genetic diversity

- Importance of genetic diversity

- What is genetic diversity?

- Measuring genetic diversity

- Extent of genetic diversity

- Low genetic diversity in threatened species and bottlenecked populations

- Variation over space and time

- Genetic differences among species

- Summary

- Further reading

- Software

- Problems

- Practical exercise: Measuring genetic diversity using microsatellites

## Chapter 4 Characterizing genetic diversity: single loci

- Describing genetic diversity

- Frequencies of alleles and genotypes

- Hardy–Weinberg equilibrium

- Expected heterozygosity

- Deviations from Hardy–Weinberg equilibrium

- Extensions of the Hardy–Weinberg equilibrium

- More than one locus: linkage disequilibrium

- Summary

- Further reading

- Software

- Problems

## Chapter 5 Characterizing genetic diversity: quantitative variation

- Importance of quantitative characters

- Properties of quantitative characters

- Basis of quantitative genetic variation

- Methods for detecting quantitative genetic variation

- Partitioning genetic and environmental variation

- Partitioning of quantitative genetic variation

- Evolutionary potential, additive variation and heritability

- Dominance variance ( $V_D$ )

Measuring genetic changes over time  
Correlations between molecular and quantitative genetic variation  
Genotype  $\times$  environment interaction  
Summary  
Further reading  
Software  
Problems

## Chapter 6 Evolutionary impacts of natural selection in large populations

The need to evolve  
Factors controlling the evolution of populations  
Selection  
Selection on quantitative characters  
Summary  
Further reading  
Software  
Problems  
Practical exercises: computer simulations

## Chapter 7 Evolutionary impacts of mutation and migration, and their interactions with selection in large populations

Factors controlling the evolution of populations  
Importance of mutation and migration and their interactions with selection in conservation  
Origin and regeneration of genetic diversity  
Mutation  
Mutation–selection balance and the mutation load  
Migration  
Migration–selection equilibria and clines  
Summary  
Further reading  
Software  
Problems

## Chapter 8 Genetic consequences of small population sizes

Importance of small populations in conservation biology  
Chance effects  
Fixation  
Effects of population bottlenecks

- Inbreeding
- Measuring population size
- Selection in small populations
- Mutation–selection equilibrium in small populations
- Computer simulation
- Summary
- Further reading
- Software
- Problems
- Practical exercises: computer simulations

## Chapter 9 Maintenance of genetic diversity

- Conservation of genetic diversity
- Fate of different classes of mutations
- Maintenance of genetic diversity in large populations
- Neutral mutations under random genetic drift
- Selection intensities vary among characters
- Balancing selection
- Reproductive fitness
- Maintenance of genetic diversity in small populations
- Summary
- Further reading
- Software
- Problems
- Practical exercises: Computer simulations

## Chapter 10 Population genomics

- Genome sequencing and population genomics
- cDNA expression microarrays
- What conservation benefits might be gained from genomics?
- Genome organization
- Insights into evolution from genomics
- Insights from gene expression studies
- Prospects for individual-locus genetic management
- Summary
- Further reading
- Software
- Problems

## **Section II Effects of population size reduction**

### **Chapter 11 Loss of genetic diversity in small populations**

Changes in genetic diversity over time

Relationship between loss of genetic diversity and reduced fitness

Effects of sustained population size restrictions on genetic diversity

Relationship between population size and genetic diversity in wild populations

Effective population size

Measuring effective population size

Gene trees and coalescence

Summary

Further reading

Software

Problems

Practical exercises: computer simulations

### **Chapter 12 Inbreeding**

What is inbreeding?

Conservation concerns with inbreeding

Measuring inbreeding: inbreeding coefficient ( $F$ )

Genetic consequences of inbreeding

Inbreeding in small random mating populations

Pedigrees

Regular systems of inbreeding

Mutation–selection balance with inbreeding

Inbreeding in polyploids

Relationships between inbreeding, heterozygosity, genetic diversity and population size

Summary

Further reading

Software

Problems

### **Chapter 13 Inbreeding depression**

Inbreeding depression in naturally outbreeding species

Inbreeding depression in the wild

Inbreeding depression due to small population size

- Inbreeding, population viability and extinction
- Characteristics of inbreeding depression
- Inbreeding depression in species that regularly inbreed
- Genetic basis of inbreeding depression
- Purging
- Detecting and measuring inbreeding depression
- Genetic rescue
- Summary
- Further reading
- Software
- Problems

## Chapter 14 Population fragmentation

- Habitat fragmentation
- Population fragmentation
- Population structure
- Completely isolated population fragments
- Measuring population fragmentation:  $F$  statistics
- Gene flow among population fragments
- Measuring gene flow
- Landscape genetics
- Impacts of different population structures on reproductive fitness
- Summary
- Further reading
- Software
- Problems

## Chapter 15 Genetically viable populations

- Shortage of space for threatened species
- How large?
- Retaining reproductive fitness
- Retaining evolutionary potential
- How large are threatened populations?
- Long-term retention of single-locus genetic diversity
- Time to regenerate genetic diversity
- Avoiding accumulation of new deleterious mutations
- Genetic goals in the management of wild populations
- Genetic goals in management of captive populations: a compromise



The fallacy of small surviving populations

Summary

Further reading

Software

Problems

### **Section III From theory to practice**

Chapter 16 Resolving taxonomic uncertainties and defining management units

Importance of accurate taxonomy in conservation biology

What is a species?

Sub-species

How do species arise?

Delineating sympatric species

Delineating allopatric species

Genetic distance

Constructing phylogenetic trees

Outbreeding depression

Defining management units within species

Summary

Further reading

Software

Problems

Practical exercise: building a phylogenetic tree

Chapter 17 Genetic management of wild populations

Genetic issues in wild populations

Increasing population size

Diagnosing genetic problems

Genetic rescue of small inbred populations by outcrossing

Genetic management of fragmented populations

Genetic issues in reserve design

Impacts of harvesting

Genetic management of species that are not outbreeding diploids

Summary

Further reading

Software

## Problems

### Chapter 18 Genetic issues in introduced and invasive species

Impact of invasive species on biodiversity

Phases in establishment of invasive species

Genetic issues in invasion biology

Evolution of native species in response to introduced species

Control of invasive species

Introgression and hybridization

Summary

Further reading

Software

Problems

### Chapter 19 Genetic management of captive populations

Why captive breed?

Stages in captive breeding and reintroduction

Founding captive populations

Growth of captive populations

Genetic management during the maintenance phase

Captive management of groups

*Ex situ* conservation of plants

Reproductive technology and genome resource banks

Managing inherited diseases in endangered species

Summary

Further reading

Software

Problems

### Chapter 20 Genetic management for reintroduction

Reintroductions

Genetic changes in captivity that affect reintroduction success

Genetic adaptation to captivity

Genetic management of reintroductions

How successful are reintroductions?

Supportive breeding

Case studies in captive breeding and reintroduction

Summary

Further reading

Software

Problems

Chapter 21 Use of molecular genetics in forensics and to understand species biology

Forensics: detecting illegal hunting and collecting

Understanding a species' biology is critical to its conservation

Population size and demographic history

Gene flow and population structure

Reintroduction and translocation

Breeding systems, parentage, founder relationships and sexing

Disease

Diet

Aging and fitness from telomere lengths

Dating using molecular clocks

Summary

Further reading

Software

Problems

Chapter 22 The broader context: population viability analysis (PVA)

What causes endangerment and extinction?

Predicting extinction probabilities: population viability analysis (PVA)

Genetics and PVA

Insights into the causes of extinction from PVA

Recovering threatened populations

Using PVA to evaluate management options: case studies

How useful are the predictions of PVA?

Lessons learned

Minimum viable population sizes (MVP)

Summary

Further reading

Software

Problems

Practical exercises: population viability analyses

*Take home messages from this book*

*Revision problems*

*Glossary*  
*References*  
*Index*

## Preface to the second edition

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Much has changed since we began the first edition of *Introduction to Conservation Genetics* 10 years ago. The human population has exceeded 6.6 billion, with consequent increased pressure on the natural world. The number of threatened species has increased by 55% to 16 306. Over the same time, global climate change has moved from being a somewhat abstract concern to perhaps the pre-eminent global political focus. The impact of climate change is now clearly discernable on the distribution and behaviour of many species. Sea-level increases are impacting on the viability of low-lying nations and the biota they contain. On a smaller scale, three-quarters of species of bears are now considered to be in danger and the Yangtze River dolphin (referred to in the first edition) has become extinct. Further, invasive species are having an ever more important impact on biodiversity, especially with rapidly increasing trade.

While our objectives in preparing this book are fundamentally those that motivated the first edition, accelerating human impacts bring into even sharper focus the need to integrate genetics into the broader conservation effort.

# Preface

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The World Conservation Union (IUCN), the leading international conservation body, recognizes the crucial need to conserve genetic diversity as one of the three fundamental levels of biodiversity. This book provides the conceptual background for understanding the role of genetic factors in extinction and managing to avoid such extinctions.

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Conservation genetics is the use of genetics to aid in the conservation of populations or species

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Conservation genetics encompasses:

- genetic management of small populations to maximize retention of genetic diversity and minimize inbreeding,
- resolution of taxonomic uncertainties and delineation of management units, and
- the use of molecular genetic analyses in forensics and to understand species' biology.

## Purpose of the book

We have endeavoured to make this book appealing to a wide readership. However it is primarily directed towards those encountering the discipline for the first time, either through formal coursework or by self-instruction.

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This book is intended to provide an accessible introduction to conservation genetics with an emphasis on general principles

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Conservation genetics is a relatively young discipline. While it is founded on more than a century of advances in evolutionary genetics, including population and quantitative genetics and plant and animal breeding, it has developed its own unique attributes, specialist journals, etc. In particular, conservation genetics focuses strongly on processes within small and fragmented populations and on practical approaches to minimize deleterious effects within them. It has implications for organizations and individuals with very different immediate concerns. These include zoo staff undertaking captive breeding programs, wildlife and fisheries biologists and ecologists, planners and managers of National Parks, reserves, water catchments and local government natural areas, foresters and farmers. Perhaps of most importance to the future, conservation genetics is of concern to a growing body of undergraduate and postgraduate students, on whom will fall much of the onus of implementing practical measures. Their enthusiasm was a major stimulus to our preparing this volume.

To make *Introduction to Conservation Genetics* accessible to this broad array of readers, we have placed emphasis on general principles, rather than on detailed experimental procedures which can be found in specialist books, journals and conference proceedings. Further, considerable attention has been devoted to clarity of presentation. We have assumed a basic knowledge of Mendelian genetics and basic statistics. Readers requiring a simpler version are referred to our *A Primer of Conservation Genetics*. Conservation genetics is a quantitative discipline as much of its strength lies in its predictions. However, we have restricted use of mathematics to simple algebra to make it accessible to a wide audience.

The material is suitable for a full tertiary course on Conservation Genetics. Further, it provides evolutionary geneticists and evolutionary ecologists with conservation examples to enthuse their students. Finally, we have endeavored to create an easily accessible and formalized reference book for both professional conservation geneticists and a wider readership.

## Précis of contents

**Chapter 1** provides an overview of the contemporary conservation context and the reasons why genetic theory and information are crucial in the management of endangered species. **Chapter 2** explores the central issue in the application of genetics to conservation biology, the relation of genetic factors to extinction risk. Inbreeding reduces reproductive capability and survival, and thereby increases extinction risk in the short term, while loss of genetic diversity reduces the long-term capacity of species to evolve in response to environmental changes.

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This book provides a broad coverage of all strands of conservation genetics

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We have divided the book into three subsequent sections: **Section I** describes the evolutionary genetics of natural populations, **Section II** explores the genetic consequences of reduced population size and **Section III** focuses on applications of genetic principles to management of threatened species in wild, semi-wild and captive situations. The relationships of genetics with broader issues in conservation biology conclude this section.



Section I (Chapters 3–10) covers essential background material in evolutionary genetics. **Chapter 3** deals with the extent of genetic diversity and methods for measuring it. Special attention is paid to comparisons of genetic diversity in threatened versus non-threatened species. **Chapters 4** and **5** describe methods and parameters used to characterize genetic diversity. As major genetic concerns in conservation biology are centred on reproduction and survival, we have placed considerable emphasis on quantitative (continuously varying) characters, as reproductive fitness is such a character (**Chapter 5**). Molecular measures of genetic diversity, for which vast data sets have accumulated, have a disturbingly limited ability to predict quantitative genetic variation. The paramount importance placed on the functional significance of genetic diversity distinguishes conservation genetics from the related field of molecular ecology, where selectively neutral variation is frequently favoured. **Chapters 6** and **7** introduce factors affecting the amount and evolution of genetic diversity in large populations. The same processes in small populations are detailed in **Chapter 8**. Chance (stochastic) effects have a much greater impact on the fate of genetic diversity in small, endangered populations than in very large populations, where natural selection has far greater influence. Since conservation genetics focuses on retention of evolutionary potential, **Chapter 9** examines the maintenance of genetic diversity. **Chapter 10** on population genomics (new to the second edition) presents important insights into genome evolution that have been revealed by whole genome sequencing and studies of gene expression using RNA expression microarrays, and considers the relevance of these new technologies to conservation management.

Having established the basic principles, **Section II** concentrates on the genetic implications of population size reduction, loss of genetic diversity (**Chapter 11**), the deleterious consequences of inbreeding on reproduction and survival (inbreeding depression) (**Chapters 12** and **13**) and the genetic effects of population fragmentation (**Chapter 14**). The section concludes with consideration of the population size required to maintain genetically viable populations (**Chapter 15**).

Section III explores practical issues, genetic resolution of taxonomic uncertainties and delineation of management units (**Chapter 16**), the genetic management of wild populations (**Chapter 17**), genetic issues relating to invasive species and their control (**Chapter 18**, new to the second edition), genetic management of captive populations (**Chapter 19**) and reintroduction (**Chapter 20**). **Chapter 21** addresses the use of molecular genetic analyses in forensics and resolution of cryptic aspects of species biology. **Chapter 22** expands to a broader picture, the integration of demographic, ecological and genetic factors in conservation biology. In particular, we explore the concepts of population viability analysis (PVA) using computer simulations. The final component, **Take home messages** presents a brief summary of the contents of the book, followed by a **Glossary**.

*Introduction to Conservation Genetics* concentrates on naturally outbreeding species of plants and animals, with lesser attention to self-fertilizing plants. Microbes receive limited coverage, as little conservation effort has been directed towards them.

We have used examples from threatened species wherever possible. However, most conceptual issues in conservation genetics have been resolved using laboratory and domesticated animals, non-threatened but related species, or by combined analyses of data sets (typically small) from many species (meta-analyses). Endangered species typically have low numbers and are often slow breeders, and it is imprudent to risk losing them through experimentation.

## **Changes in the second edition**

Since we began the first edition 10 years ago, there have been major changes to the discipline, both to threat status and to the underlying science. The number of threatened species has increased overall, the most spectacular increase being the 15-fold increase in threatened amphibians, due primarily to a fungal disease and global warming. As a consequence of global climate

change, many species will need to move to remain in their favoured climatic envelope and may need to be translocated for this to happen. There has been steady progress in most areas of conservation genetics, but population genomics has arisen since the first edition. Further, invasive species have an increasing role as threatening agents and research on them has expanded dramatically. Overall, the field of conservation genetics has developed rapidly, as judged by number of journal papers, number of courses and new methodologies. Further, the sources of information have diversified with the rapid rise of databases and websites. Software tools for statistical analyses and computer simulations have also proliferated.

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The second edition has been extensively updated and revised to encompass the major advances in conservation genetics since we wrote the first edition, but the general format has been retained

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We have been heartened by the favourable reception to the first edition both as a textbook and as a professional reference book. We have preserved the main organization in this second edition, but updated the content and references throughout and added two new chapters. New topics include material on population genomics, the implications of genomics to conservation genetics, a box on meta-analysis, landscape genetics, proposals for DNA-based taxonomy, DNA barcoding, decline in haplo-diploids due to loss of genetic diversity at the sex locus, the Jurassic Park scenario, the impact of transgenics and genetic issues in invasive species. We have also expanded the coverage of adaptive changes in response to global climate change. The number of references has expanded by 48% in line with the expansion of the field and over half of the references are new to this edition. We have added references to software packages, important websites and databases, as they are now widely available and extensively used. The index

has been improved by doubling the number of entries and by using italic numbers for tables and bold ones for figures and illustrations. In spite of the new material, the overall length is similar to that of the first edition. This has been achieved by streamlining the presentation and by omitting a few topics of lesser importance.

## Format

The book is profusely illustrated to make it visually attractive and to tap the emotional commitment that many feel to conservation. To highlight significant points and make it easy to review, the **main points** of each chapter are given in a box at the start of the chapter along with **Terms** used in the chapter. A **Summary** is given at the end of each chapter. Within chapters, the **main points** of each section are highlighted in small boxes. Much of the information is presented in figures (~220), as we find that biology students respond better to those than to information in text or tables. For some figures, the message is highlighted in italics in the caption. Numerous examples and case studies have been used to illustrate the application of theory to real-world conservation applications. These have been chosen to be motivating and informative to our audience. Case studies are given in **boxes** throughout the book. Boxes are also used to provide additional information in a way that does not impede the flow of information for those who wish to skip such detail.

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Extensive effort has been made to motivate readers by making the book attractive, interesting, informative and easy to follow

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We are deeply indebted to Karina McInnes, whose elegant drawings add

immeasurably to our words. She has drawn 20 new illustrations for the second edition and new illustrations drawn for the *Primer* are also used.

The revision of the textbook has been aided by comments from several cohorts of students at Macquarie University and from many colleagues and students throughout the world. We thank these people for their contributions.

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The order of topics both within and across chapters has been designed to motivate students

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The order of topics throughout the book, and within chapters, is based on our teaching experience. We have chosen to introduce practical conservation issues as early as possible, with the details of parameter estimation etc. provided later. We hope that readers will find it more stimulating to appreciate *why* a parameter is important, before understanding *how* it is derived. As an example, [Chapter 2](#) directly addresses the relationship between genetics and extinction, and provides an overview of much of the later material, prior to a detailed treatment of inbreeding in [Chapters 12](#) and [13](#).

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Each chapter has been designed to provide instructors with material suitable for one lecture, along with additional information for independent study

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In presenting material, we have aimed for a balance between that necessary for student lectures, and a comprehensive coverage for advanced students and conservation professionals. The material in each chapter is more than adequate for a single lecture, allowing instructors to choose what they wish to emphasize in their course, but the material in each chapter should not prove overwhelming to students. Some topics are too extensive for a single lecture. We have therefore divided evolution in large populations into two chapters. Chapters are designed to be comprehensible on a 'stand-alone' basis, so there is some repetition of material, but this is reduced compared to the first edition.

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Worked examples and problems with solutions are provided

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Everyone who has taught genetics recognizes that mastery of the discipline comes through active participation in problem-solving, rather than passive absorption of 'facts'. Consequently, worked **Examples** are given within the text for most equations presented. **Problem** questions are posed at the end of each chapter, together with **Revision problems** at the end of the book. Problem answers are given on the Cambridge University Press website [www.cambridge.org/9780521702713](http://www.cambridge.org/9780521702713).

Named species are used in many problem questions, to make them more realistic. These are usually fictitious problems, but reflect situations similar to those that have, or reasonably might have, occurred in the named species. Real data are referenced where used.

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Practical exercises are suggested for several chapters

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**Practical exercises** are suggested at the end of chapters covering topics where laboratory exercises are relevant. Most of these have been trialled in our own teaching and are frequently computer exercises, using readily available software.

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For clarity and brevity, referencing is mainly restricted to reviews and recent papers

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References are given to reviews and recent papers, these being sufficient to gain access to the most significant literature. Space does not permit direct reference to many other excellent studies by our colleagues. An annotated **General bibliography**, relevant to many chapters, is given at the end of [Chapter 1](#). Readers seeking further detail on specific topics will find an annotated list of suggested **Further reading** at the end of each chapter. We have also included a sprinkling of books written for popular audiences to provide an introduction to some of the, often controversial, characters involved in conservation biology, and the passions that motivate their work. Referencing and data presentation are more extensive for contentious topics.

As most of the principles of conservation genetics apply equally to different eukaryotic species, we primarily use common names in the text. Genus and species names in the **index** are cross-referenced to common names.

## Controversies

The development of conservation genetics has been driven by what many consider to be a global environmental crisis – ‘the sixth extinction’. As a consequence, many other dimensions, economic, political, social, ethical and emotional, impact upon the field. The fate of species, populations and habitats are in the balance. We have flagged these controversies and attempted to provide a balanced, up-to-date view, based upon information available in late 2007. Where feasible, we have consulted experts to corroborate facts and interpretations. Inevitably, some readers will disagree with some of our views, but we trust that they will accept that alternative interpretations are honestly given. New data may alter perspectives and some such changes have occurred since the first edition.

We hope that readers find the book as stimulating to read as we found it to write, but not as tiring! Feedback, constructive criticism and suggestions will be appreciated (email: [dick.frankham@mq.edu.au](mailto:dick.frankham@mq.edu.au)).

We maintain a website with updated information, corrections, etc. at <http://www.cambridge.org/9780521702713>.

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texts as models in our preparation of this book. RF thanks Stuart Barker for his highly influential roles as undergraduate lecturer, Ph.D. supervisor, mentor and collaborator.

Our book could not have been written without the efforts of the students, staff and collaborators in the RF–DAB laboratory.

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## Copyright acknowledgments

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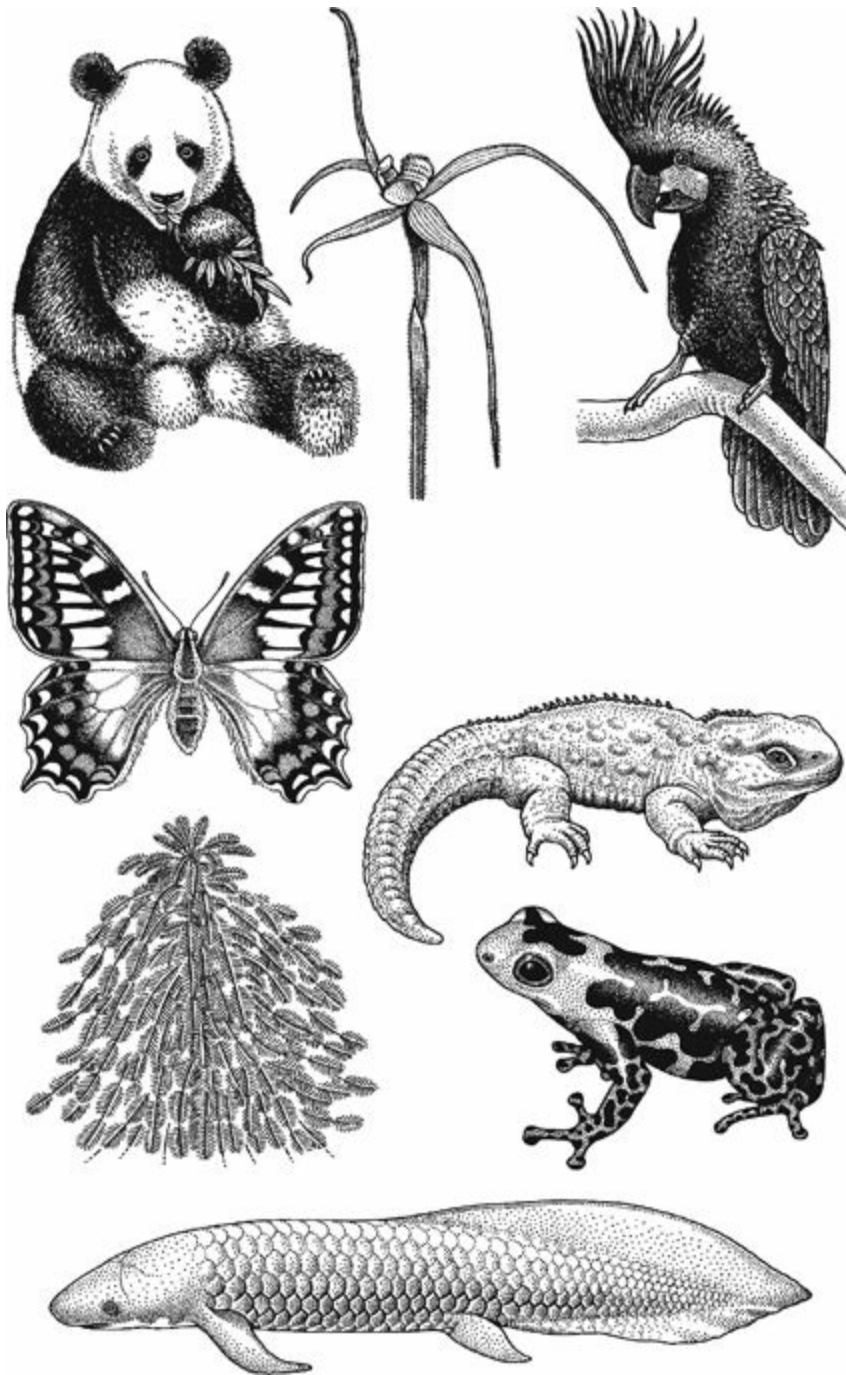
# Chapter 1 Introduction

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Many species are threatened with extinction. Populations of endangered species typically decline due to habitat loss, over-exploitation, introduced species, pollution and climate change. At small population sizes, additional factors (demographic and environmental variation, genetics and catastrophes) increase their risk of extinction. Conservation genetics applies genetic knowledge to reduce the risk of extinction in threatened species

## Terms

Biodiversity, bioresources, catastrophes, critically endangered, demographic stochasticity, ecosystem services, endangered, environmental stochasticity, evolutionary potential, extinction vortex, forensics, genetic diversity, genetic drift, genetic stochasticity, inbreeding depression, introgression, meta-analysis, purging, reproductive fitness, speciation, stochastic, threatened, vulnerable



Selection of threatened species. Clockwise: panda (China), an Australian orchid, palm cockatoo (Australia), tuatara (New Zealand), poison arrow frog (South America), lungfish (Australia), Wollemi pine (Australia) and Corsican swallow-tail butterfly

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## The ‘sixth extinction’

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Biological diversity is rapidly being depleted as a consequence of human actions

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The current extinction crisis has been called the ‘sixth extinction’, as its magnitude compares with that of the other five mass extinctions revealed in the geological record (Leakey & Lewin [1995](#)). Extinction is a natural part of the evolutionary process, species typically persisting for ~5–10 million years. Biodiversity (the variety of ecosystems, species, populations within species, and genetic diversity within species) is maintained when extinctions are balanced by the origin of new species (**speciation**). However, mass extinctions, such as the cosmic cataclysm at the end of the Cretaceous, 65 million years ago, reduce biodiversity. It takes many millions of years for recovery. The sixth extinction is equally dramatic. Species are being lost at a rate that far exceeds the origin of new species but, unlike previous mass extinctions, this is mainly due to human activities (IUCN [2007](#)).

Conservation genetics, like all components of conservation biology, is motivated by the need to preserve biodiversity and to reduce current rates of extinction.

## Why conserve biodiversity?

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Maintenance of biodiversity is justified for four reasons: the economic value of bioresources, ecosystem services, aesthetics and rights of living organisms to exist

**Bioresources** include all of our food, many pharmaceutical drugs, natural fibres, rubber, timber, etc. Their value is many billions of dollars annually. For example the world fish catch is valued at \$US58 billion annually (UNEP 2007). Over half of the top 150 prescription drugs in the USA contain active ingredients derived directly or indirectly from living organisms (Millennium Ecosystem Assessment 2005a). Malaria, one of the world's most deadly diseases, has been treated with drugs derived from natural products, including quinine, chloroquine, mefloquine, doxycycline and artemisins. Further, the natural world contains many novel, potentially useful resources (Beattie & Ehrlich 2004). Ants synthesize novel antibiotics that are being investigated for use in human medicine, spider silk is stronger weight-for-weight than steel and may provide the basis for light high-tensile fibres, etc.

**Ecosystem services** are essential biological functions that are provided free of charge by living organisms (Millennium Ecosystem Assessment 2005a). Examples include oxygen production by plants, climate regulation, carbon sequestration, nutrient cycling, natural pest and disease control and pollination of crop plants. In 2000, these services were valued at \$US38 trillion ( $10^{12}$ ) per year, similar in size to the yearly global national product (Balmford *et al.* 2002). For example, honeybee pollination of crops has been valued at \$US2 billion per annum (UNEP 2007). The benefit : cost ratio for wildlife conservation versus exploitation is 100 : 1, based on a review of 200 case studies (Balmford *et al.* 2002).

Humans derive pleasure (aesthetic value) from living organisms, expressed in cultivation of ornamental plants, keeping pets, visiting zoos and nature



reserves, ecotourism and viewing wildlife documentaries. This translates into direct economic value. For example, the aggregate revenue generated by ecotourism in Southern Africa was estimated as \$US3.66 billion in 2000, roughly 50% of all tourism revenue in the area (Millennium Ecosystem Assessment [2005a](#)).

The ethical justification for biodiversity conservation is that one species on Earth does not have the right to drive others to extinction, parallel to abhorrence of genocide among human populations.

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IUCN recognizes the need to conserve biodiversity at three levels: genetic diversity, species diversity and ecosystem diversity

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The primary international conservation body, IUCN (the World Conservation Union), recognizes the need to conserve the biological diversity at all three levels (McNeely *et al.* [1990](#)). Genetics is a key consideration at all levels, being the sole issue in the first, having an important role in species viability, and a role in ecosystem viability (Bangert *et al.* [2005](#); Lankau & Strauss [2007](#)). Its importance to ecosystem viability has only recently been documented. For example, a seagrass community with higher genetic diversity recovered better following a heatwave, and snails and isopods associated with seagrass also benefited (Reusch *et al.* [2005](#)).

## **Endangered and extinct species**

### **Recorded extinctions**

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Over 900 extinctions have been documented since records began in 1600, the majority of these being island species

The proportions of species in different groups known to have gone extinct since records began in 1600 are small, being only 1–2% in mammals and birds (Table 1.1). However, the rate of extinction has generally increased with time (Primack 2006) and many species are now threatened. Further, many extinctions must have occurred unnoticed, especially those due to habitat loss (Millennium Ecosystem Assessment 2005a).

**Table 1.1** *Recorded extinctions, 1600 to present, for mainland and island species worldwide*

Taxa	Number of extinctions on				% extinctions on islands	% of taxon extinct
	Islands	Mainlands	Oceans	Total		
Mammals <sup>a</sup>	51	30	4	85	60	2.1
Birds <sup>a</sup>	92	21	0	113	81	1.3
Reptiles <sup>a</sup>	20	1	0	21	95	0.3
Amphibians <sup>a</sup>	0	2	0	2	0	0.05
Fish <sup>a</sup>	1	22	0	23	4	0.1
Molluscs <sup>b</sup>	151	40	0	191	79	
Invertebrates <sup>a</sup>	48	49	1	98	49	0.01
Flowering plants <sup>a</sup>	139	245	0	384	36	0.2

<sup>a</sup> Primack (1998).

<sup>b</sup> WCMC (1992).

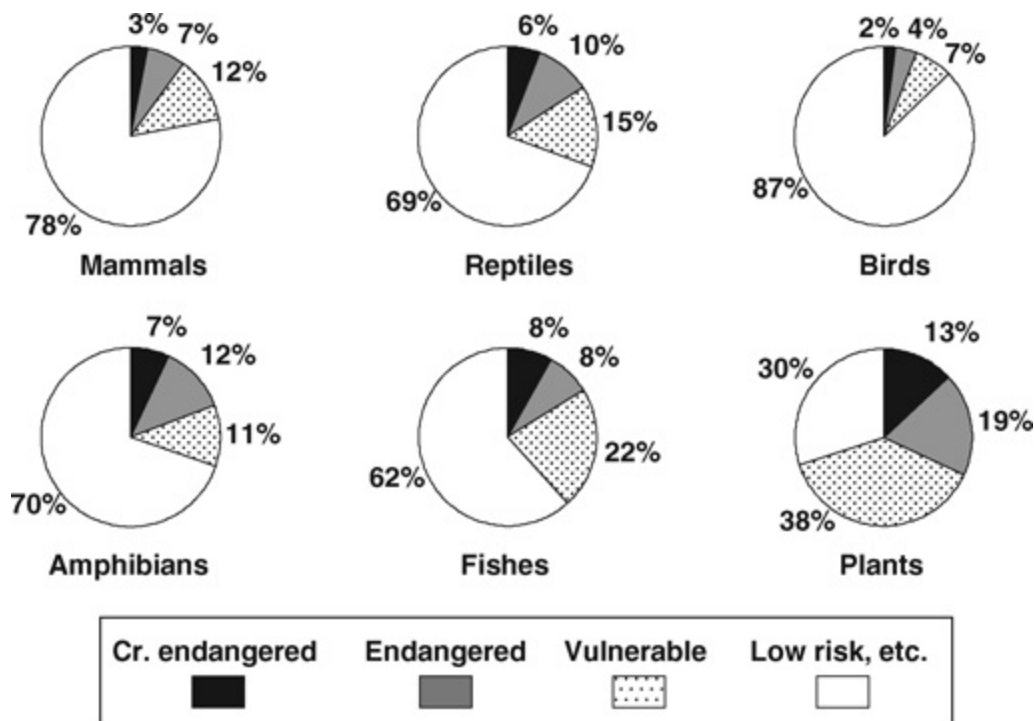
The majority of recorded extinctions, and a substantial proportion of currently threatened species, are on islands (Table 1.1). For example, 81% of all recorded bird extinctions are insular, four-fold greater than the proportion of bird species found on islands (Myers 1979). We will return to vulnerability

and significance of insular populations later in the book.

## Extent of endangerment

Many species are threatened with extinction, including 23% of vertebrate animals, 51% of invertebrates and 70% of plant species

IUCN (the World Conservation Union) defines as **threatened** species with a high risk of extinction within a short time frame. These species fall into the categories of critically endangered, endangered and vulnerable, as defined below. In mammals, reptiles, birds, amphibians, fish and plants IUCN (2007) classified 22%, 31%, 13%, 30%, 38% and 70% of assessed species as threatened (Fig. 1.1). Of mammal species, 1.4% are extinct, 0.1% extinct in the wild, 3.4% critically endangered, 7.2% endangered and 12.0% vulnerable.



**Fig. 1.1** Percentages of mammals, birds, reptiles, amphibians, fishes and plants categorized as critically endangered, endangered, vulnerable and at lower risk (after IUCN [2007](#)).

There are considerable uncertainties about the data for all except mammals, birds, amphibians and gymnosperms, as the status of many species has not been assessed in the other groups. Estimates for microbes are not available as the number of extant species in these groups is unknown.

## Projected extinction rates

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Projections indicate greatly elevated extinction rates in the near future

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There is a consensus that extinction rates are destined to accelerate markedly, typically by 100–1000-fold or more above the ‘normal’ background extinction rates deduced from the fossil record (Millennium Ecosystem Assessment [2005a](#)). This is primarily due to the continuing escalation of the human population, and its anticipated impact on the rest of the global biota.

Current extinction rates are 10 times those in the fossil record (UNEP [2007](#)). In line with these projections, the overall threat status of the planet's birds has worsened since 1988 (Butchart *et al.* [2004](#)). Further, there has been an alarming recent decline in amphibians, probably exacerbated by a pathogenic fungus in association with global warming. Up to 122 amphibian species have disappeared since 1980 (Pounds *et al.* [2006](#), [2007](#)). Global climate change is projected to commit 15–37% of species to extinction by 2050 (Thomas, C. D. *et al.* [2004](#)). In Britain 28% of native plant species have declined over the last 40 years, 54% of native bird species have declined over

the last 20 years and 71% of native butterflies have declined over ~20 years (Thomas, J. A. *et al.* 2004).

## What is an endangered species?

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Endangered species are those with a high risk of immediate extinction

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The IUCN (2007) has defined criteria to classify threatened species into **critically endangered**, **endangered**, **vulnerable** and **lower risk**, based on population biology principles developed largely by Mace & Lande (1991). These categories are defined in terms of the rate of decline in population size, restriction in habitat area, the current population size and/or the probability of extinction (Table 1.2). A critically endangered species exhibits at least one of the characteristics described in the second column under A–E in Table 1.3. These characteristics include: an 80% or greater decline in population size over the last 10 years (or three generations); an extent of occupancy of less than 100 square kilometres; a population size of less than 250 mature adults; a probability of extinction of 50% or more over 10 years (or three generations). For example, there are only about 65 Javan rhinoceroses surviving in Southeast Asia and their numbers are continuing to decline, so this species falls into the category of critically endangered. Other examples are given in the Problems at the end of the chapter.

**Table 1.2** *Defining endangerment (IUCN 2007 criteria)*

Category	Probability of extinction	Time
Critically endangered	50%	10 yrs or 3 generations
Endangered	20%	20 yrs or 5 generations
Vulnerable	10%	100 yrs

**Table 1.3** *Information used to decide whether species fall into the critically endangered, endangered or vulnerable IUCN categories (simplified from IUCN 2007).* A species falling within any of the categories A–E in the critically endangered column is defined as critically endangered. Similar rules apply to endangered and vulnerable

Criteria (any one of A–E)	Critically endangered	Endangered	Vulnerable
A Actual or projected decline in population size and continuing threat	80% over the last 10 years or 3 generations	50%	30%
B Extent of occurrence: or area of occupancy of: and any two of: (i) severely fragmented or known to exist at: (ii) continuing declines, and (iii) extreme fluctuations	<100 km <sup>2</sup> <10 km <sup>2</sup> a single location	<5000 km <sup>2</sup> <500 km <sup>2</sup> ≤5 locations	<20 000 km <sup>2</sup> <2000 km <sup>2</sup> ≤10 locations
C Population numbering and an estimated continuing decline	<250 mature individuals	<2500	<10 000
D Population estimated to number:	<50 mature individuals	<250	<1000
E Quantitative analysis showing a probability of extinction in the wild of:	at least 50% within 10 yrs or 3 generations, whichever is the longer	20% in 20 yrs or 5 generations	10% in 100 yrs

There are similar, but less extreme characteristics required to categorize species as endangered, or vulnerable. Species falling outside these criteria are designated as lower risk. IUCN has also defined categories of extinct, extinct in the wild, conservation dependent, near threatened and data deficient (IUCN 2007).

While many other systems are used throughout the world to categorize endangerment, the IUCN provides the only international system for listing of species in the IUCN *Red Books* of threatened species (de Grammont & Cuarón 2006; IUCN 2007). Rankings obtained using the IUCN classifications are related, but not identical to rankings from two other widely used systems, and all are related to extinction risks determined using computer models (O'Grady *et al.* 2004). We primarily use the IUCN system throughout this book.

## Importance of listing

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Listing a species or sub-species as endangered provides a scientific foundation for national and international legal protection, and may lead to remedial actions for recovery

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Endangerment is the basis for legal protection for species. For example, most countries have Endangered Species Acts that provide legal protection for threatened species and usually require the formulation of recovery plans. Threatened species are also protected from trade by the 172 countries that have signed the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2007). This provides important protection for approximately 28 000 species of plants and 5000 species of animals, including threatened cats, primates, whales, parrots, reptiles, amphibians, fish, etc.

## What causes extinctions?

### Human-associated factors

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The primary factors contributing to extinction are habitat loss, introduced species, over-exploitation, pollution and climate change. These factors are caused by humans, and related to human population growth

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The primary factors contributing to extinction are directly or indirectly related to human impacts and to human population size. The human population has grown exponentially, reaching 6.6 billion in late 2007. The last 1.5 billion increase (30%) occurred in only about 20 years. By 2050, the population is projected to rise to 7.8–10.8 billion (United Nations Population Division [2007](#)). Consequently, human impacts on wild animals and plants will continue to worsen.

### **Additional threats in small populations**

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Additional demographic, environmental, catastrophic and genetic factors increase the risk of extinction in small populations

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Human-related activities often reduce species to population sizes where they are susceptible to additional environmental, catastrophic, demographic or genetic factors. These factors are discussed extensively throughout the book. Even if the original cause of population decline is removed, problems associated with small population size will still persist.

**Environmental stochasticity** is random unpredictable variation in



environmental factors, such as rainfall and food supply. **Demographic stochasticity** is random variation in birth and death rates and sex-ratios due to chance alone. **Catastrophes** are extreme environmental events such as tornadoes, floods, harsh winters, disease epidemics, etc.

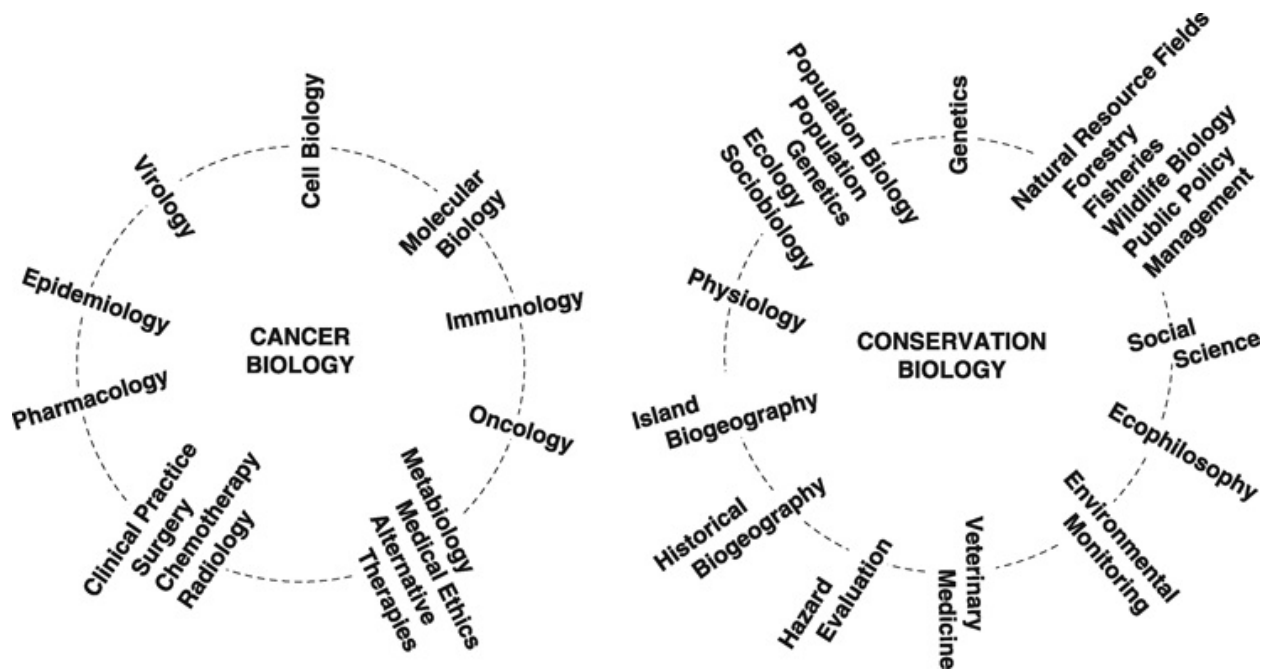
**Genetic factors** encompass the deleterious impacts on species of inbreeding, loss of genetic diversity and the accumulation of deleterious mutations. **Inbreeding** (the production of offspring from related parents), on average, reduces birth rates and increases death rates in the inbred offspring (**inbreeding depression**: [Chapters 12](#) and [13](#)). **Genetic diversity** is the raw material upon which natural selection acts to bring about adaptive evolutionary change. Consequently, loss of genetic diversity reduces the ability of populations to adapt to changing environments ([Chapters 6](#), [8](#) and [11](#)).

Environmental and demographic stochasticity and the impact of catastrophes interact with inbreeding and genetic diversity in their adverse effects on populations ([Chapters 2](#) and [22](#)). If populations become small for any reason, they become more inbred, further reducing population size and generating additional inbreeding. Smaller populations also lose genetic diversity and suffer reductions in their ability to adapt to changing environments. This feedback between reduced population size, loss of genetic diversity and inbreeding is referred to as the **extinction vortex**. The complicated interactions between genetic, demographic and environmental factors can make it extremely difficult to identify the immediate cause(s) of any particular extinction event.

## **Recognition of genetic factors in conservation biology**

Sir Otto Frankel, an Austrian-born Australian, was largely responsible for recognizing the importance of genetic factors in conservation biology, beginning with papers in the early 1970s (Frankel [1970](#), [1974](#); see Soulé & Frankham [2000](#) for biographical information). Subsequently, Frankel strongly influenced Michael Soulé of the USA and collaborated with him on

the first conservation book that clearly discussed the role of genetic factors (Frankel & Soulé 1981). Soulé is recognized as the ‘father’ of modern conservation biology, having been instrumental in founding the Society for Conservation Biology, serving as its first President, and participating in the establishment of *Conservation Biology*, the premier journal in the field. Throughout the 1980s, Soulé had a profound influence on the development of conservation biology as a multidisciplinary crisis field, drawing on ecology, genetics, wildlife biology and resource biology (Fig. 1.2).



**Fig. 1.2** Structure of conservation biology and the position of genetics in it (after Soulé 1985). *Conservation biology is a crisis discipline akin to cancer biology, to which it is compared.*

## What is conservation genetics?

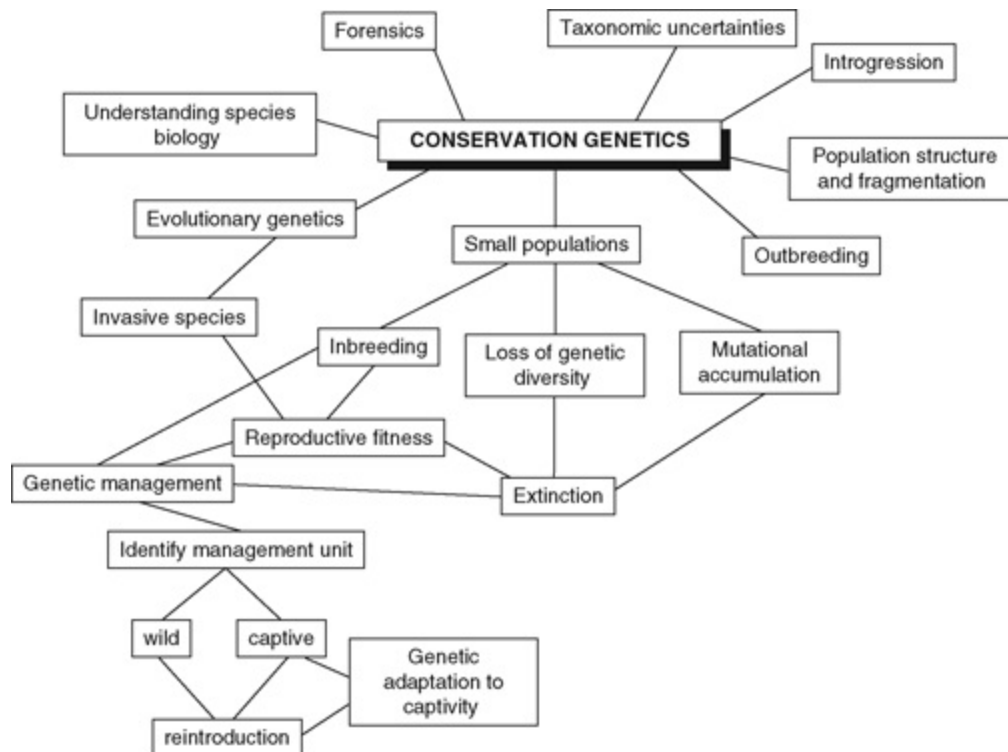
Conservation genetics aims to minimize the risk of extinction

Conservation genetics encompasses the use of genetic theory and techniques to reduce the risk of extinction in threatened species. Its longer-term goal is to preserve species as dynamic entities capable of coping with environmental change. Conservation genetics is derived from evolutionary genetics and from the quantitative genetic theory that underlies selective breeding of domesticated plants and animals. However, these theories generally concentrate on large populations where the genetic constitution of the population is governed by predictable deterministic factors. Conservation genetics is now a discrete applied discipline focusing on the genetic consequences arising from reduction of once large, outbreeding populations to small units where stochastic factors and the effects of inbreeding are paramount.

This textbook addresses the major issues in the field, including:

- the deleterious effects of **inbreeding** on reproduction and survival (**inbreeding depression**)
- loss of **genetic diversity** and consequent reduced ability to evolve in response to environmental change (loss of **evolutionary potential**)
- fragmentation of populations and reduction in gene flow
- random processes (**genetic drift**) overriding natural selection as the main evolutionary process
- accumulation and loss (**purging**) of deleterious mutations
- genetic adaptation to captivity and its adverse effects on reintroduction success
- evolutionary processes in invasive species and their impacts on threatened species
- resolving taxonomic uncertainties
- defining management units within species
- deleterious effects on fitness that sometimes occur as a result of outcrossing (**outbreeding depression**)
- use of molecular genetic analyses in **forensics** and to understand aspects of species biology important to conservation.

The structure and content of conservation genetics is illustrated in Fig. 1.3.



**Fig. 1.3** Structure and content of conservation genetics.

## Examples of the use of genetics to aid conservation

Knowledge of genetics aids conservation in the following ways.

### Reducing extinction risk by minimizing inbreeding and loss of genetic diversity

Due to a long period of small population size, the endangered Florida panther was affected by several genetic problems, including low genetic diversity and inbreeding-related defects (poor sperm and physical abnormalities). These effects have been alleviated by introducing individuals from its most closely related sub-species in Texas ([Chapter 17](#)). Captive populations of many endangered species (e.g. golden lion tamarin) are managed to minimize loss of genetic diversity and inbreeding ([Chapter 19](#)).



Florida panther

## **Identifying species or populations at risk due to reduced genetic diversity**

Asiatic lions exist in the wild only in a small population in the Gir Forest in India. This population has a very low level of genetic diversity, indicating it has a severely compromised ability to evolve ([Chapter 11](#)), as well as being susceptible to demographic and environmental risks ([Chapter 22](#)). The recently discovered Wollemi pine, an Australian relict species previously known only from fossils, exists as a small population with no genetic diversity among individuals. Its extinction risk is extreme. All individuals that were tested were susceptible to a common dieback fungus. Its management involves keeping the site secret and quarantined, plus the propagation of plants in other locations and the commercial sale of plants throughout the world.



Wollemi pine

## Resolving fragmented population structures

Information regarding the extent of gene flow among populations is critical to determine whether a species requires human-assisted exchange of individuals to prevent inbreeding and loss of genetic diversity ([Chapter 14](#)). Wild populations of the red-cockaded woodpecker are fragmented, causing genetic differentiation among populations and reduction in genetic diversity in the smaller populations. Consequently, part of the management of this species involves moving (translocating) individuals between populations to minimize inbreeding and to maintain genetic diversity.



Red-cockaded woodpecker

## Resolving taxonomic uncertainties

The taxonomic status of many invertebrates and lower plants is unknown ([Chapter 16](#)). Thus, an apparently widespread and low-risk species may, in reality, comprise a complex of distinct taxa, some rare or endangered. Molecular genetic studies have shown that Australia is home to well over 100 locally distributed species of velvet worms (*Peripatus*) rather than the seven widespread morphological species previously recognized. Even African elephants have been shown to consist of at least two, rather than one species.



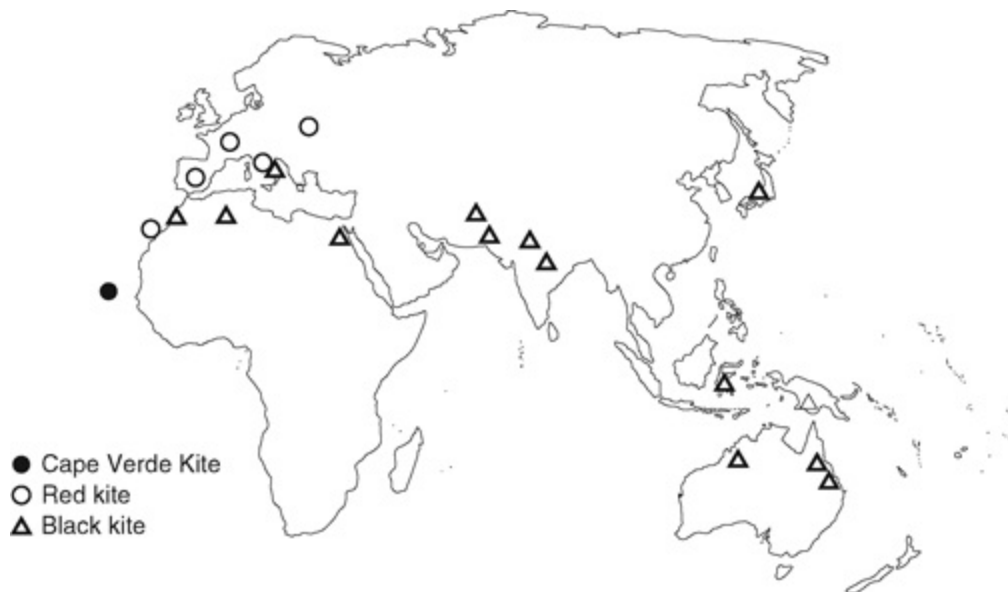
Velvet worm

Equally, genetic markers may reveal that previously identified threatened species are misdiagnosed and undeserving of their threatened status. Molecular genetic analyses have shown that the threatened Cape Verde kite living on islands 500 km off the coast of West Africa are genetically indistinguishable from the non-endangered and widespread black kite (see [Box 1.1](#)). Similarly, the endangered colonial pocket gopher from Georgia is indistinguishable from the common pocket gopher in that region.

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**Box 1.1 Use of molecular genetic analyses to resolve the controversial taxonomic status of the Cape Verde kite**

(Johnson et al. 2005)

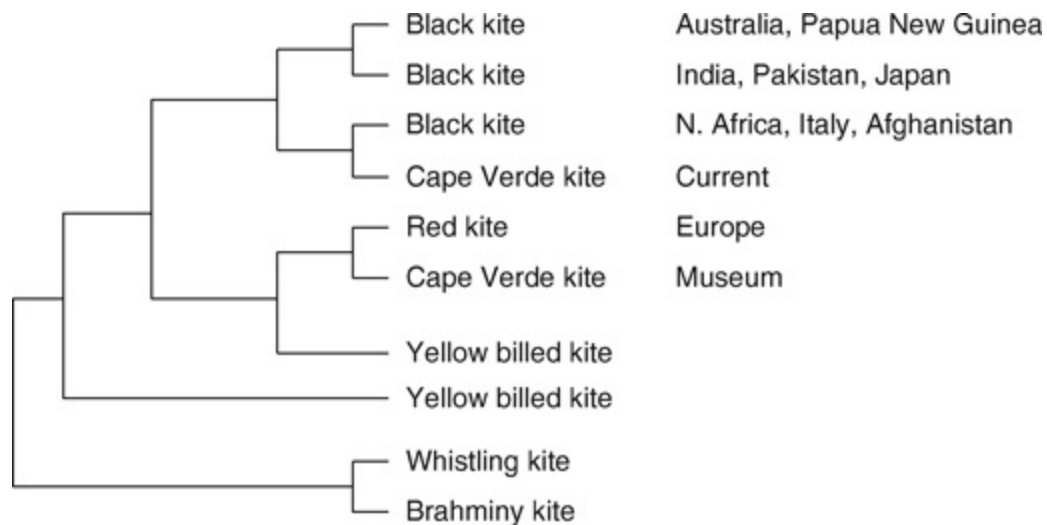


The Cape Verde kite from the Cape Verde Islands 500 km off the coast of Senegal in West Africa is one of the rarest birds of prey in the world. However, its distinctiveness from widespread and non-endangered red and black kites was a matter of controversy. To resolve this issue, DNA was extracted from 43 individuals from known species and sub-species of kites from 27 geographical locations, plus seven museum specimens of Cape Verde kites collected between 1897 and 1924 and five kites trapped on Cape Verde Islands in 2000. Phylogenetic trees constructed from the DNA sequence data (see [Chapter 16](#)) revealed that red and black kites were distinct and non-overlapping. The museum specimens of Cape Verde kites all lay within the red kite grouping. Conversely, the recently trapped birds from Cape Verde Island grouped within the black kites. Neither the museum specimens of Cape Verde kites nor the recently captured ones were distinct species. Thus, the case for conserving the Cape Verde kite as a distinct species is not supported.





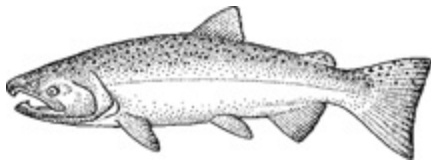
Cape Verde kite



## Defining management units within species

Populations within species are sometimes adapted to somewhat different environments, and they may justify management as separate units ([Chapter](#)

16). Their hybrids may be at a disadvantage, sometimes even displaying partial reproductive isolation. For example, coho salmon (and many other fish species) display genetic differentiation among populations from different river systems. These populations show evidence of adaptation to different conditions (morphology, swimming ability and age at maturation). Thus, they should be managed as separate populations (Small *et al.* 1998).



Coho salmon

## Detecting hybridization

Many rare species of plants, salmonid fish and canids are threatened with being ‘hybridized out of existence’ by crossing with common species (Chapter 18). Molecular genetic analyses have shown that the critically endangered Ethiopian wolf (simian jackal) is subject to hybridization with local domestic dogs.

## Non-intrusive sampling for genetic analyses

Many species are difficult to capture, or are badly stressed in the process. DNA can be obtained from hair, feathers, sloughed skin, faeces etc. in non-intrusive sampling, the DNA amplified and genetic studies completed without disturbing the animals (Chapter 3). For example, the critically endangered northern hairy-nosed wombat is a nocturnal burrowing marsupial that can only be captured with difficulty. Sampling has been achieved by placing adhesive tape across the entrances of their burrows to capture hair when the animals exit or enter. DNA from non-invasive sampling can be used to identify individuals, census populations, determine mating patterns and population structures, and measure levels of genetic diversity (Chapter 21).



Northern hairy-nosed wombat

## **Defining sites for reintroduction**

Molecular analyses may provide additional information on the historical distribution of species, expanding possibilities for conservation action. For ecological reasons, reintroductions should preferentially occur within a species' historical range. The northern hairy-nosed wombat exists in a single population of approximately 100 animals at Clermont in Queensland, Australia. DNA samples obtained from museum skins identified an extinct wombat population at Deniliquin, hundreds of kilometres away in New South Wales as also belonging to this species. Thus, Deniliquin is a potential site for reintroduction ([Chapter 21](#)). Similarly, information from genotyping DNA from sub-fossil bones has revealed that the endangered Laysan duck previously existed on islands other than its present distribution in the Hawaiian Islands.

## **Forensics**

Molecular genetic methods are widely applied to provide forensic evidence for litigation. These include the detection of illegal hunting and collection. Sale for consumption of meat from threatened whales has been detected by analysing whale meat in Japan and South Korea. Mitochondrial DNA sequences showed that about 9% of the whale meat on sale came from protected species of whales, rather than from the minke whales that can be taken legally ([Chapter 21](#)). Methods have also been devised to identify species of origin using small amounts of DNA from shark fins, tiger bones and elephant ivory.



Humpback whale

## Understanding species biology

Many aspects of species biology that are important in conservation can be determined using molecular genetic analyses (see [Chapter 21](#)). For example, mating patterns and reproduction systems are often difficult to determine in threatened species. Studies using genetic markers established that loggerhead turtle females mate with several males. Mating systems in many plants have been established using genetic markers. Birds are often difficult to sex, resulting in several cases where two birds of the same sex were placed together to breed. Molecular genetic methods are now available to sex birds without having to resort to surgery. Paternity can be determined in many species, including chimpanzees. Endangered Pyrenean brown bears are nocturnal and secretive. Methods have been devised to census and sex these animals, based upon hair and faeces.

Dispersal and migration patterns are often critical to species survival prospects. These are difficult to determine directly, but can be inferred by examining the geographic distribution of genetic variations.

Each of these issues will be explored further in later chapters.

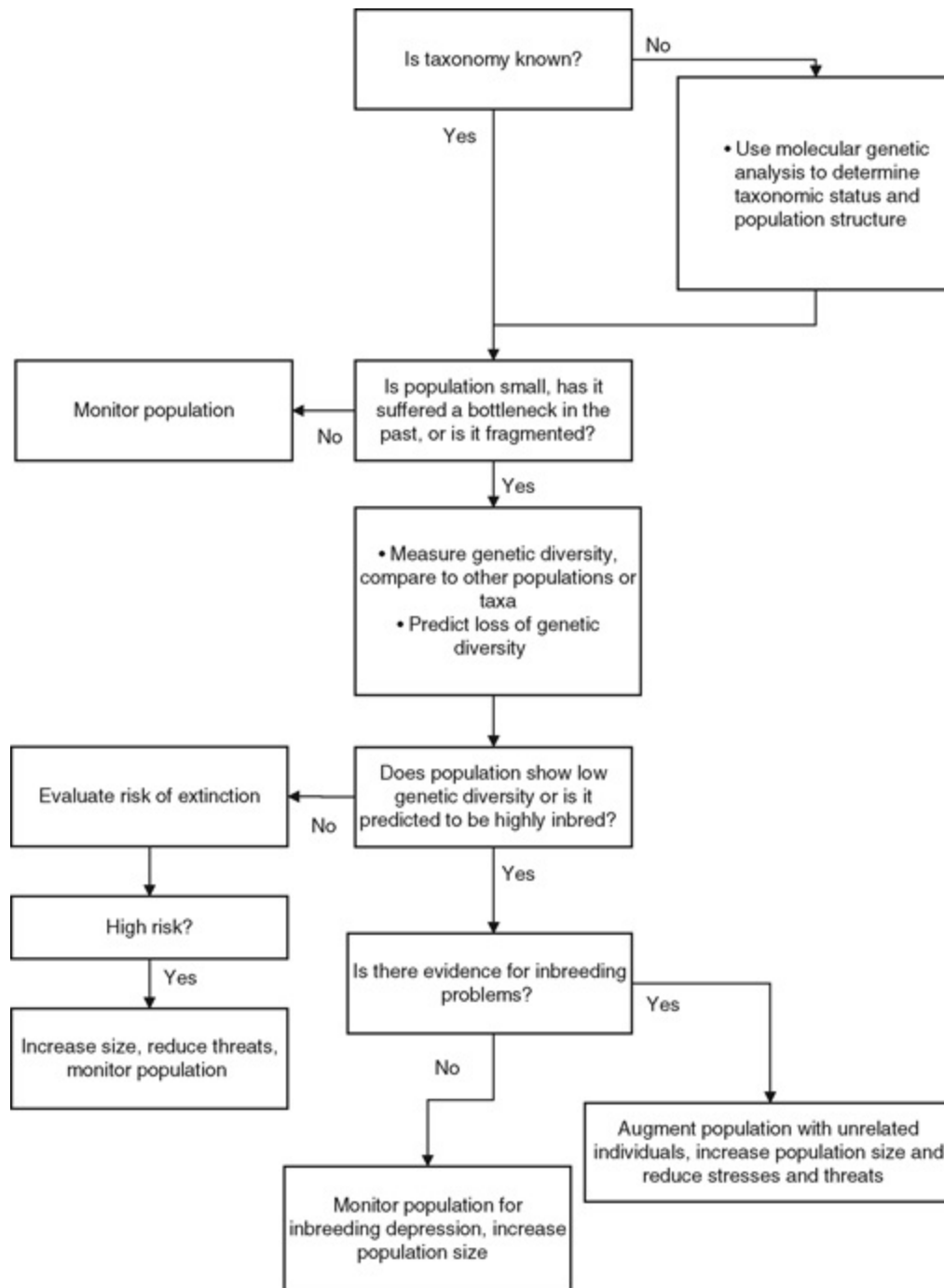
## Genetic management of threatened species

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Three primary questions are asked when seeking to manage endangered species: Is the taxonomy known? Is the population small and/or fragmented, or likely to become so? Are all essential aspects of species biology known?

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A flowchart of a sample of the information required for the genetic management of threatened populations is given in [Fig. 1.4](#). The first crucial group of questions relate to whether the taxonomy is clearly known. If not, this can often be resolved using genetic marker studies ([Chapter 16](#)). A related question is whether populations within species differ sufficiently to justify separate management, as would typically be accorded to sub-species.



**Fig. 1.4** Flowchart of a sample of questions that are asked in relation to genetic issues in conservation.

The second group of critical questions relates to population size, and how that impacts the genetic characteristics of the population. We have already indicated that the species population size and the extent of fragmentation are

central to an assessment of extinction risk through genetic factors. These questions are addressed in Section II of the textbook.

The third question (not shown) relates to whether all the critical aspects of the species' biology are known and whether unknown parameters can be resolved using molecular genetic analyses. These issues and others are all treated in Section III of the book, 'From theory to practice'.

## Methodology in conservation genetics

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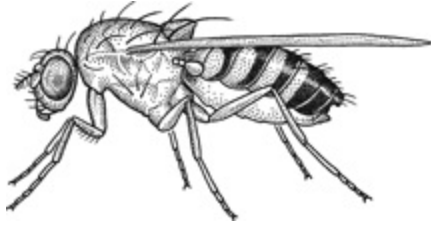
Conceptual issues in conservation genetics are typically resolved using experiments with laboratory species, computer simulations and combined analyses of data from many wildlife species (meta-analyses)

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Information on endangered species is often limited and these species are typically unsuitable for experimental studies. Advances in conservation genetics typically come from the interplay of theory, computer simulations and experimentation, operating in a feedback loop. This is analogous to approaches used in climate modelling. The traditional means for testing theory and resolving issues are to carry out replicated experiments with controls. As endangered species are unsuitable for doing this, we have turned to laboratory species such as fruit flies, flour beetles and mice.

For example, inbreeding has been found to result in deleterious effects on reproduction and survival (**reproductive fitness**) in essentially all naturally outbreeding populations of laboratory animals, domestic animals, domestic plants and wild species that have been adequately investigated (see [Chapter 13](#)). While some might question the use of surrogate laboratory species to

study conservation genetics, we are aware of no case where studies with laboratory species have yielded qualitative results at variance with those found for other species with similar breeding systems.



Fruit fly

Computer simulations provide means for examining complex models with many interacting factors. Mathematical models and computer simulation have provided much of the theory that we apply to the management of small populations. Computer simulation has an extremely important role in **population viability analysis** (PVA). This determines extinction risk due to the combined effects of demographic and environmental factors, catastrophes and genetic factors ([Chapters 2 and 22](#)).



Data sets for endangered species are typically small, so conclusions from any one species are often not convincing. Consequently, statistical analyses frequently combine data from a variety of species or populations in **meta-analyses** ([Box 2.4](#)). For example, the impact of inbreeding on captive mammals was evaluated by combining data on juvenile survival of inbred versus outbred offspring from 44 populations of mammals ([Chapter 12](#)).



Much of the supporting evidence in this book comes from laboratory studies, computer simulations and meta-analyses. However, we have sought to use examples from endangered populations wherever adequate evidence could be found.

## Sources of information

An annotated list of widely used references is given at the end of this chapter. References within the text are primarily reviews and recent papers that provide an entry into each topic area. The primary research information appears in the specialist journal *Conservation Genetics*, in general conservation biology journals (*Conservation Biology*, *Animal Conservation* and *Biological Conservation*), in more broadly based evolutionary biology journals (including *Molecular Ecology*, *Evolution*, *Journal of Heredity*, *Heredity*, *Genetics*, *American Naturalist*, *PLoS Genetics* and *Genetical Research*), and in prestigious general science journals (such as *Nature*, *Science*, *Proceeding of the National Academy of Sciences of the USA*, *Proceedings of the Royal Society of London B* and *PLoS Biology*). An increasing number of websites have valuable information and databases, especially those for the Millennium Ecosystem Assessment and IUCN *Red List* (referenced below).

## Summary

1. The biodiversity of the planet is rapidly being depleted due to direct and indirect human actions.
2. An endangered species is one with a high risk of extinction within a short time.
3. The primary factors contributing to population declines are habitat loss, introduced species, over-exploitation, pollution and climate change. In small populations, additional accidental (stochastic) demographic and environmental factors, catastrophes and genetic processes increase the risk of extinction.
4. Conservation genetics is the use of genetics to aid in conservation and

minimize the risk of extinction.

5. Genetics contributes to conservation through minimizing inbreeding and loss of genetic diversity, identifying populations of concern, determining population structure, resolving taxonomic uncertainties, defining management units within species, identifying populations and sites for reintroductions, using molecular genetic analyses in forensics and by providing tools to improve our understanding of species biology.

## General bibliography

(For full bibliographical details see References)

Allendorf & Luikart (2006) *Conservation and the Genetics of Populations*. Authoritative textbook covering similar topics to this one.

Aise (2004) *Molecular Markers, Natural History and Evolution*. Readable textbook concerned with molecular population genetics, determination of life history parameters and taxonomy and brief consideration of conservation genetics.

CITES: Convention on International Trade in Endangered Species of Wild Fauna and Flora. The website describes the agreement, the countries that have signed and the species covered by the convention <http://cites.org/>

Falconer & Mackay (1996) *Introduction to Quantitative Genetics*. Outstanding textbook on population and quantitative genetics, with an emphasis on animal and plant breeding. Similar level to this textbook.

Fox & Wolf (eds.) (2006) *Evolutionary Genetics: Concepts and Case Studies*. Recent reviews on many issues relevant to conservation genetics.

Frankham *et al.* (2004) *A Primer of Conservation Genetics*. A brief (~200

page) introductory textbook on conservation genetics.

Groom *et al.* (2006) *Principles of Conservation Biology*. Basic textbook in conservation biology, with a reasonable coverage of genetic issues.

Hartl & Clark (2007) *Principles of Population Genetics*. Basic textbook in population genetics with a strong molecular flavour.

Hedrick (2005a) *Genetics of Populations*. Thorough and authoritative textbook in population genetics.

IPCC (2007a, b, c) *Climate Change 2007*. Authoritative three-volume consensus review on climate change from the Intergovernmental Panel on Climate Change who shared the 2007 Nobel Peace Prize. Free access available at [www.ipcc.ch/](http://www.ipcc.ch/)

IUCN (2007) *Red List of Threatened Species*. Website with full details of the internationally recognized IUCN categorization system for designating threatened species, plus listings of threatened species. <http://www.iucnredlist.org/>

Primack (2006) *Essentials of Conservation Biology*. Basic textbook in conservation biology with a brief coverage of genetic issues.

Quammen (1996) *The Song of the Dodo*. An interesting and stimulating book written for a general audience.

UNEP (2007) *The Global Environmental Outlook 4*. Authoritative report on status of the planet; see especially chapter 5 on biodiversity [www.unep.org/geo/geo4/](http://www.unep.org/geo/geo4/)

## Problems

These problems are designed to review assumed knowledge on Mendelian inheritance, probability and statistics (1.1–1.8), and to evaluate your ability to place species into the IUCN categories of risk (1.9–1.12).

- 1.1 Mendelian inheritance. If two parents have genotypes of  $A_1A_2$  and  $A_1A_2$  at a locus, what are the expected proportions of the different progeny genotypes?
- 1.2 Mendelian inheritance. What are the expected proportions of the different progeny genotypes produced from the cross between  $A_1A_2 B_1B_2$  and  $A_1A_2 B_1B_2$  parents, assuming independent assortment of the A and B loci?
- 1.3 Mendelian inheritance. What does independent assortment of two gene loci imply about the location of the two loci?
- 1.4 Transcription and translation. Given the strand of DNA below, insert the complementary strand, the mRNA, the tRNA anticodon and the amino acids specified.

Complementary DNA

Coding DNA strand

TAC TTT GGG ATT

mRNA

tRNA anticodon

Amino acids

- 1.5 Statistics. A pair of mice produces 50 males and 80 females in their lifetime. Use a  $\chi^2$  test to determine whether this differs from a 1 : 1 ratio.
- 1.6 Probability. What are the possible ratios of females to males in families of size 4? What are their respective probabilities of occurrence?
- 1.7 Statistics. The four phenotypes in an  $F_2$  were found in the numbers 100 : 20 : 35 : 5. Use a  $\chi^2$  test to determine whether these differ from the 9 : 3 : 3 : 1 expectation.
- 1.8 Statistics. Ten golden lion tamarin females have litter sizes of 0, 1, 2, 3, 2, 1, 0, 2 and 3. What is the mean and standard deviation of litter

size?

- 1.9 IUCN categories. In what IUCN category would you place the northern hairy-nosed wombat? It exists as a relatively stable population of approximately 100 individuals (say 45 adults) in one small area in Queensland, Australia.
- 1.10 IUCN categories. In what IUCN category would you place the southern bluefin tuna? Its population size has declined by almost 90% over the last 30 years, from 3.7 million in 1965 to 423 000 in 1994.
- 1.11 IUCN categories. In what IUCN category would you place the thylacine, a marsupial carnivore from Tasmania, Australia? It has not been seen in the wild since 1933, and the last animal in captivity died in 1936.
- 1.12 IUCN categories. In what IUCN category would you place Attwater's prairie chicken? It occurs in Texas, USA and had a total population size of 456 in 1993, distributed over three fragmented populations (372, 24 and 60 individuals) that may have been isolated since 1937. The species once numbered about 1 million, and has recently been declining at a rate of about 5% per year.

## **Practical exercises: Categorizing endangerment of species**

Provide students with groups of three species (one abundant, and two falling into different threatened categories – see examples below) and ask them to categorize them using the IUCN system. Supply students with suitable reference materials.

Gray wolf, Puerto Rican parrot, northern right whale  
Deer mouse, whooping crane, golden lion tamarin  
Red kangaroo, orange-bellied parrot, koala  
Indian mynah bird, giant panda, Javan rhinoceros  
African buffalo, Mauritius pink pigeon, chimpanzee  
European starling, Iberian lynx, Mediterranean monk seal

## Chapter 2 Genetics and extinction

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Inbreeding and loss of genetic diversity are unavoidable in small populations of threatened species. They reduce reproduction and survival in the short term, diminish the capacity of populations to evolve in response to environmental change in the long term, and thereby increase extinction risk

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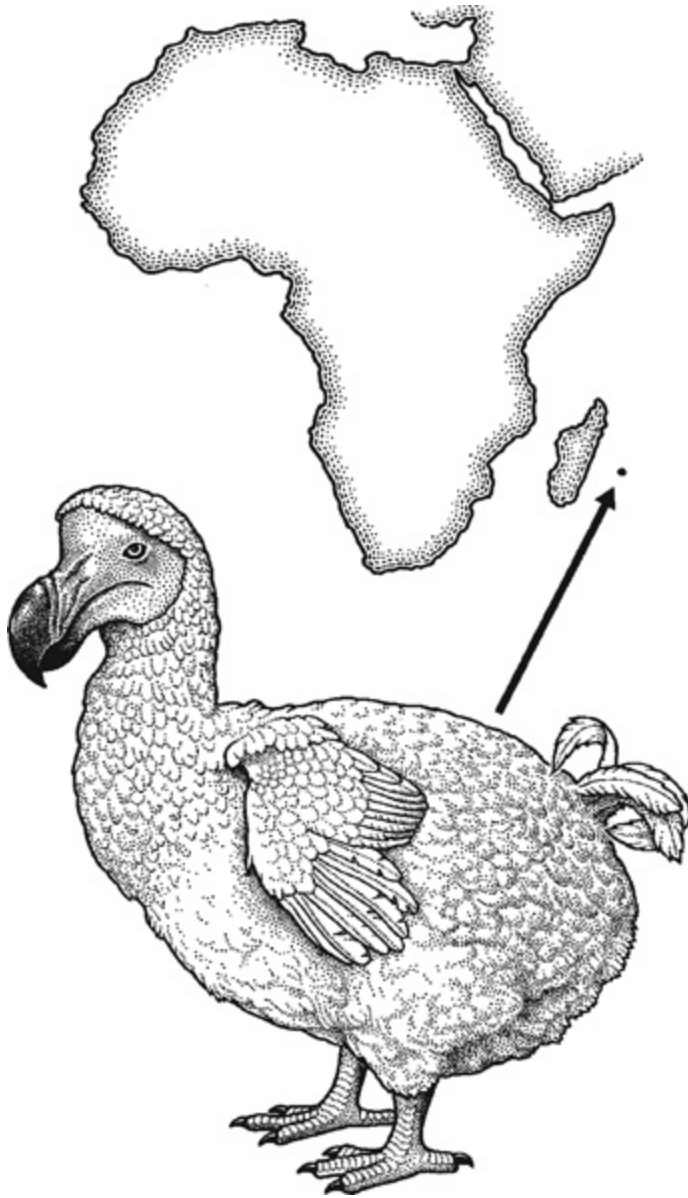
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### Terms

Endemic, extinction vortex, genetic diversity, genetic drift, inbreeding, inbreeding coefficient, major histocompatibility complex (MHC), meta-analysis, outbreeding, self-incompatibility, statistical power

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Extinct dodo and its previous distribution on the island of Mauritius

## Genetics and the fate of endangered species

Until recently, the contribution of genetic factors to the fate of endangered species was controversial and generally considered to be minor. Lande (1988) summarized this opinion by suggesting that demographic and environmental fluctuations (stochasticity), and catastrophes, would cause extinction before genetic deterioration became a serious threat to wild populations. However, there is now a compelling body of theoretical and empirical evidence that genetic processes in small populations are intimately involved with their fate (Frankham 2005). Specifically:

- many surviving populations have now been shown to be genetically compromised (they have reduced genetic diversity and are inbred)
- inbreeding causes extinctions in deliberately inbred experimental populations of non-endangered species
- inbreeding has contributed to extinctions in some natural populations and there is circumstantial evidence to implicate it in many other cases
- computer projections based on real life histories (including demographic, environmental and catastrophic factors) show that inbreeding causes elevated extinction risks in realistic situations faced by natural populations
- loss of genetic diversity increases the susceptibility of populations to extinction, especially when environmental conditions are changing
- genetic factors have time to impact most species before they are driven to extinction
- outcrossing has been shown to lead to recovery in many inbred populations (**genetic rescue**).

If genetic factors are ignored, extinction risk will be underestimated and inappropriate recovery strategies may be used for threatened species.

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Inbreeding reduces reproduction and survival



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**Inbreeding** is the production of offspring from individuals related by descent, e.g. cousin, brother–sister, parent–offspring matings, self-fertilization, etc. (Box 2.1 and Chapter 12). Inbreeding reduces reproduction and survival (reproductive fitness) of offspring, an effect referred to as **inbreeding depression**. For example, inbred individuals showed higher juvenile mortality than outbred individuals in 41 of 44 captive mammal populations studied by Ralls & Ballou (1983). On average, brother–sister mating resulted in a 33% reduction in juvenile survival (Ralls *et al.* 1988). By extrapolation, it was anticipated that inbreeding would increase the risk of extinction in wild populations. Further, the impact of inbreeding depression was expected to be greater in the wild than in captivity, as natural environments are generally harsher.

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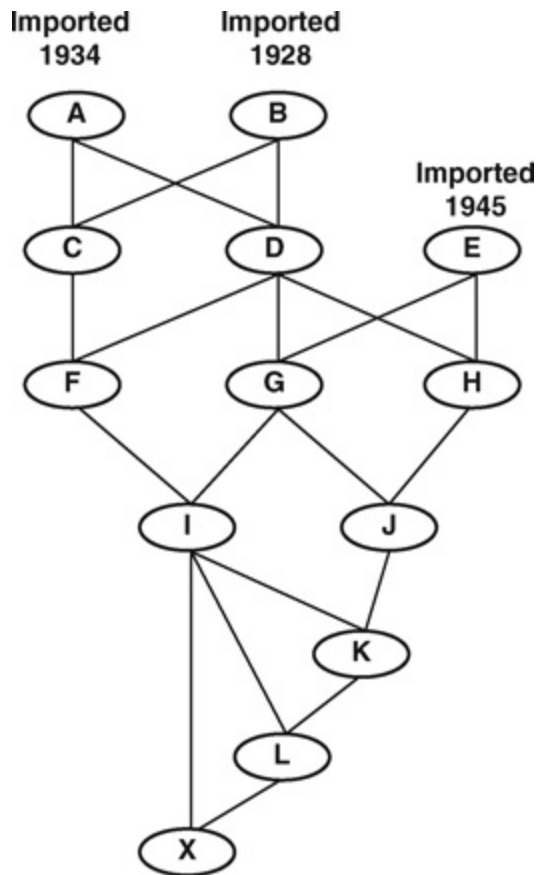
### Box 2.1 Measures of inbreeding

Inbreeding is the production of offspring from related individuals. There are repeated matings of relatives in the pedigree below of a Nigerian giraffe X born in Paris Zoo in 1992 (Bingaman-Lackey 1999). This highly inbred calf died three weeks after birth.

#### The inbreeding coefficient ( $F$ )

The inbreeding coefficient of an individual refers to how closely related its parents are. When parents are unrelated, offspring  $F = 0$ , while, for completely inbred individuals  $F = 1.0$ . Levels of inbreeding in offspring for different kinds of relationships among parents are:

Parents	Offspring $F$
Unrelated	0
Brother–sister, mother–son, or father–daughter	0.25
Half-brother–half-sister (half-sibs)	0.125
First cousins	0.0625
Self-fertilization (or selfing)	0.5



Inbreeding accumulates in closed populations (those without immigration) and complete inbreeding can eventually arise from repeated inbred matings; an  $F$  of 0.999 is reached after 10 generations of self-fertilization, while an  $F$  of 0.986 occurs after 20 generations of brother-sister mating. The Nigerian giraffe X above had an inbreeding coefficient of 0.52 (see Problem 12.11).

Levels of inbreeding can be determined from pedigrees, or inferred from heterozygosities for genetic markers ([Chapters 4 and 12](#)).

There is now clear evidence that inbreeding adversely affects most wild populations. For example, inbred individuals showed inbreeding depression in 141 cases (90%) out of 157 valid data sets from natural situations (Crnokrak & Roff [1999](#)). Results were similar across birds, mammals,

poikilotherms and plants in this meta-analysis involving 34 species ([Chapter 13](#); [Box 2.4](#) below). Species exhibiting inbreeding depression in the wild include: mammals (Florida panthers, golden lion tamarins, gray wolves, lions, Mexican wolves, native mice, shrews and Soay sheep), birds (greater prairie chicken, Mexican jay, song sparrow, red-cockaded woodpecker and reed warbler), fish (Atlantic salmon, desert topminnow and rainbow trout), a reptile, a snail, an insect (butterfly) and many species of plants (see Frankham [1995a](#); Keller & Waller [2002](#); Liberg *et al.* [2005](#); Pimm *et al.* [2006](#); Hedrick & Frederickson [2008](#)).



Nigerian giraffe

Genetic rescue of inbred populations has been achieved by outcrossing in many species, including bighorn sheep, deer mice, Florida panthers, gray wolves, lions, Mexican wolves, greater prairie chickens, Swedish adders, desert topminnow fish, several species of plants and many populations of laboratory and domestic species (Tallmon *et al.* [2004b](#); Frankham [2005](#); Hogg *et al.* [2006](#); Pimm *et al.* [2006](#); Bossuyt [2007](#); Hedrick & Frederickson [2008](#); Trinkel *et al.* [2008](#)).

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Genetic diversity is required for populations and species to evolve in response to environmental change

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**Genetic diversity** is the extent of heritable variation in a population, or species ([Chapters 3–5](#)). Genetic diversity is required for populations to evolve in response to environmental change ([Chapters 6, 8](#) and [11](#)). Such environmental change is a ubiquitous feature of life on Earth. Consequently, if there is no genetic diversity in a population or species, it is likely to become extinct in response to any major environmental change. Genetic diversity is lost by sampling effects in small random mating populations at the same time as they become inbred, so the two processes are closely related ([Chapters 8](#) and [11–13](#)).

## Relationship between inbreeding and extinction

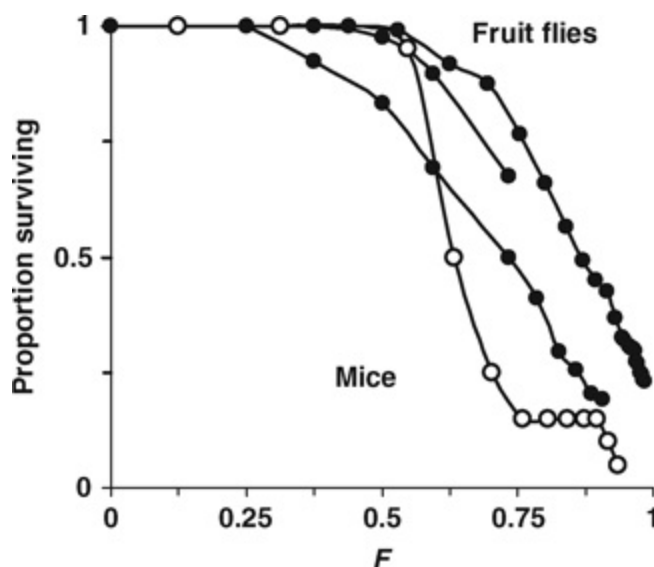
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Deliberately inbred populations of laboratory and domestic animals and plants show greatly elevated extinction rates

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Between 80% and 95% of deliberately inbred populations died out after eight generations of brother–sister mating or three generations of self-fertilization (Frankel & Soulé [1981](#)). In Japanese quail, 338 populations inbred by continued brother–sister mating were all extinct after four generations. Such extinctions could have been due to inbreeding,

demographic stochasticity (random fluctuations in birth and death rates and sex-ratios), or a combination of these effects. However, under circumstances where demographic stochasticity can be ruled out, inbreeding clearly increased the risk of extinction in captive populations (Frankham 2005). Examples from mice and fruit flies are shown in Fig. 2.1. While extinctions do not begin until intermediate levels of inbreeding have been reached, this does not indicate that lower levels of inbreeding are benign, only that offspring per pair must drop from large numbers (over 100 in the fruit flies) to below 2 for extinctions to occur.



**Fig. 2.1** Relationship between inbreeding and extinction. Populations of mice and two species of fruit flies (one with two populations) were inbred using brother–sister matings (Frankham 1995b). Demographic stochasticity made very little or no contribution to these extinctions. *The proportions of populations going extinct rises with inbreeding.*

## All major taxonomic groups suffer inbreeding depression

Inbreeding depression affects mammals, birds, reptiles, amphibians, fish, invertebrates and plants

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Most studies report similar inbreeding depression for naturally outbreeding species across major diploid taxa. Inbreeding depression for wild populations of homeotherms, poikilotherms and plants do not differ significantly (Crnokrak & Roff [1999](#)). Nor are there significant differences in inbreeding depression under captive conditions among mammalian orders, or across vertebrates (Ralls *et al.* [1988](#); Wilcken [2002](#)).

Inbreeding depression in plants is typically higher for gymnosperms than angiosperms (Husband & Schemske [1996](#)). This could be related to a higher level of polyploidy (more than two doses of each chromosome, e.g.  $4n$  vs.  $2n$ ) in the latter than the former. Since the rate of increase in homozygosity is slower in polyploids than in diploids, polyploids are expected to suffer somewhat less inbreeding depression (see [Chapter 13](#)).

## **Inbreeding and extinction in the wild**

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Inbreeding elevates extinction risks in wild populations

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Three lines of evidence indicate that inbreeding elevates the extinction risk of wild populations:

- direct evidence that inbreeding and loss of genetic variation contribute to the extinction of populations in nature
- computer projections show that inbreeding will increase extinction risks for wild populations, and
- most species are not driven to extinction before genetic factors impact them.

## Extinctions in wild populations due to inbreeding and loss of genetic diversity

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Three studies have demonstrated the involvement of inbreeding in extinctions of natural populations

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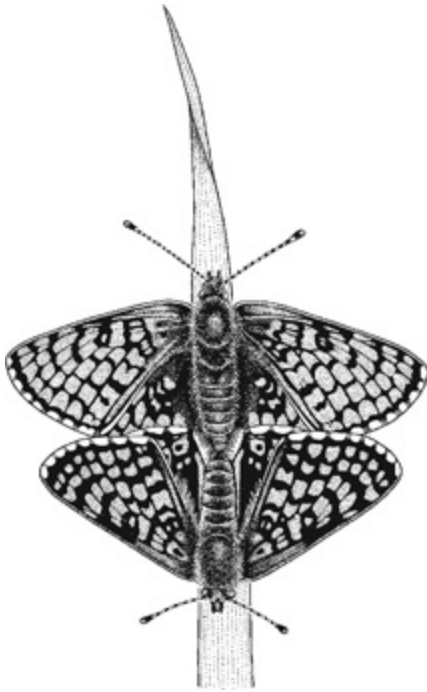
Inbreeding was a significant predictor of extinction risk for butterfly populations in Finland after the effects of all other ecological and demographic variables had been removed ([Box 2.2](#)). Similarly, experimental populations of evening primroses founded with low levels of genetic diversity (and higher inbreeding:  $F = 0.08$ – $0.09$ ) exhibited 69% extinction rates over three generations in the wild, while populations with lower inbreeding ( $F = 0.04$ ) showed only a 25% extinction rate (Newman & Pilon [1997](#)). Thus, a very small difference in inbreeding level translated into a large difference in extinction rate. Another plant study has documented adverse impacts of inbreeding on extinction in wild populations of the shore campion in Spain (Vilas *et al.* [2006](#)).

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### **Box 2.2 Inbreeding and extinction risk in butterfly populations in Finland (Saccheri *et al.* [1998](#); Nieminen *et al.* [2001](#)).**

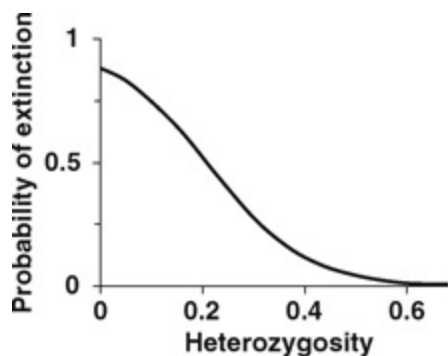
Using molecular genetic analyses, levels of heterozygosity were determined in 42 butterfly populations in Finland in 1995, and their extinction or survival recorded in the following year. Thirty-five survived to autumn 1996 and seven went extinct. Extinction rates were higher for populations with lower heterozygosity, an indication of inbreeding, even

after accounting for the effects of demographic and environmental variables (population size, time trend in population size and area) known to affect extinction risk. The curve in the margin represents the relationships between extinction probability and proportion of loci heterozygous for average sized populations.



Glanville fritillary butterfly

The causal link between inbreeding and extinction risk was confirmed by producing full-sib inbred and outbred groups of butterflies and placing them in the field. All six inbred populations went extinct over one breeding and over-wintering cycle, whilst four of six outbred populations persisted.





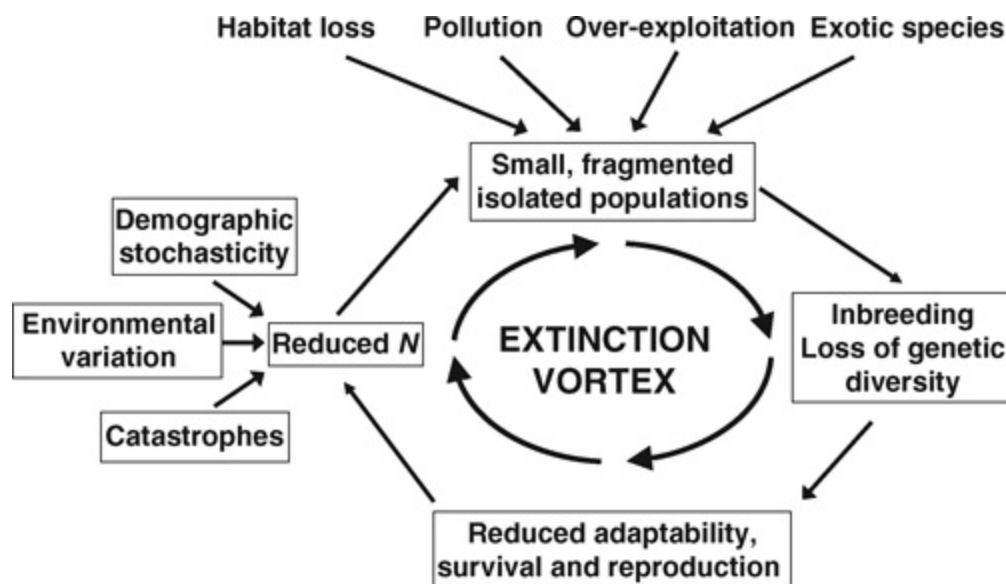
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## The extinction vortex

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The effects of inbreeding depression and loss of genetic diversity frequently interact with demographic, environmental and catastrophic factors in an 'extinction vortex'

If populations become small for any reason (human impacts, demographic or environmental stochasticity, or catastrophes), they become more inbred and less demographically stable, further reducing population size and increasing inbreeding. This feedback cycle is referred to as the **extinction vortex** (Fig. 2.2). Notably, the complicated interactions between genetic, demographic and environmental factors can make it extremely difficult to identify the immediate cause(s) for any particular extinction event (see Chapter 22).



**Fig. 2.2** The extinction vortex. This describes the possible interactions

between human impacts, inbreeding, loss of genetic diversity and demographic instability in a downward spiral towards extinction.

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Small populations are more likely to suffer from extinctions than large populations for both genetic and ecological reasons

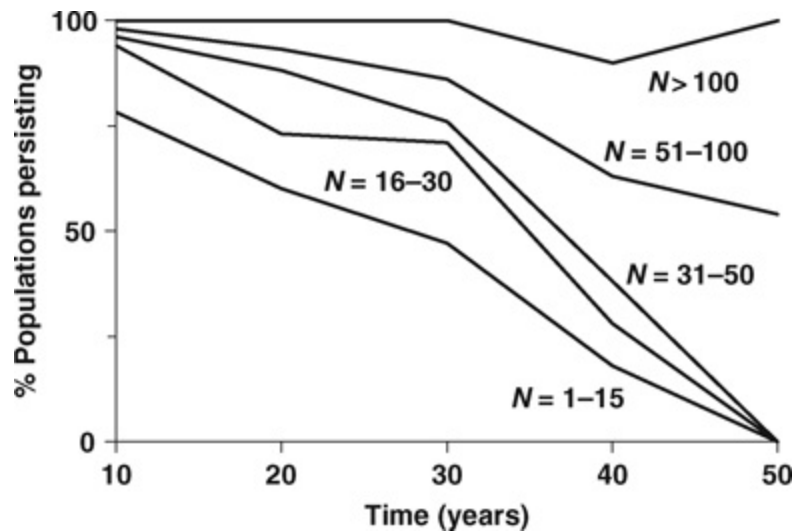
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Smaller populations are expected to be more prone to extinction than larger ones for demographic, ecological and genetic reasons. Berger (1990) found a strong relationship between population size and persistence in North American bighorn sheep (Fig. 2.3). All populations of <50 became extinct within 50 years. A wide variety of demographic, ecological and genetic factors may have contributed to these extinctions, with inbreeding depression and loss of genetic diversity being among those considered most likely. In a related vein, mammalian extinctions in national parks in western North America were related to park area, and thus to population sizes (Newmark 1995). Extinctions were more frequent for populations with smaller initial population sizes, larger fluctuations in population size and shorter generation times. As we will see later, all of these effects are predicted by genetic considerations (Chapters 11–13), but most are also expected from demographic and environmental considerations.



Bighorn sheep



**Fig. 2.3** Relationship between persistence and population size in North American bighorn sheep (after Berger 1990). *Extinction rates are higher in smaller than larger populations.*

Declines in population size or extinction in the wild have been attributed, at least in part, to inbreeding in many populations, including bighorn sheep, Florida panthers, greater prairie chickens, heath hens, middle spotted woodpeckers, adders, wolf spiders, plants and many island species (Frankham 2005; Reed *et al.* 2007).

There is circumstantial evidence that inbreeding has contributed to many other wildlife extinctions

## Extinction proneness of island populations

The majority of extinctions of plants and animals have been of island species, although these represent a minority of all species

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Recorded extinctions since 1600 reveal that a majority of extinctions have been of island forms, even though island species represent a minority of total species in all groups ([Table 1.1](#)). Further, substantial proportions of species listed as endangered and vulnerable are insular ([Chapter 1](#)).

Human factors have been the major causes of extinction on islands over the past 50 000 years. These human impacts have typically driven down population sizes to points where stochastic factors come into play and extinctions typically result from the combined impacts of all deterministic and stochastic factors.

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Circumstantial evidence points to inbreeding and loss of genetic diversity contributing to the extinction proneness of island populations of many species

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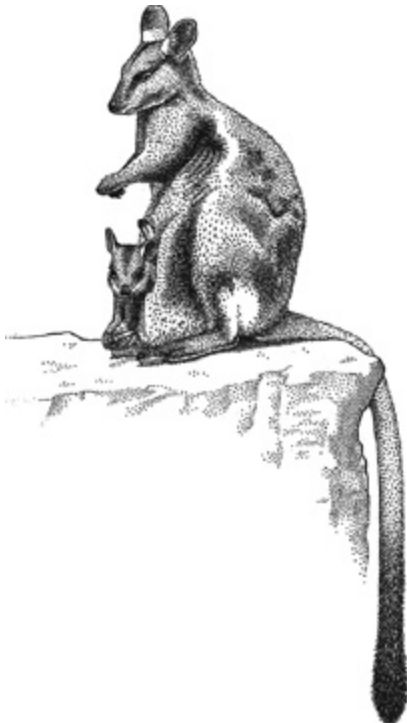
Island populations typically are smaller, have less genetic diversity and are more inbred than mainland populations. Many island populations are inbred to levels where captive populations show an elevated risk of extinction (Frankham [1997](#), [1998](#)). For example, black-footed rock wallabies on Barrow Island off the west coast of Australia have very low levels of genetic diversity

and exhibit evidence of inbreeding depression ([Box 2.3](#)). Further, euros (a kangaroo species) on the same island also have reduced genetic diversity and suffer from chronic anaemia and higher parasite loads than their mainland counterparts (M. D. B. Eldridge *et al.* pers. comm.).

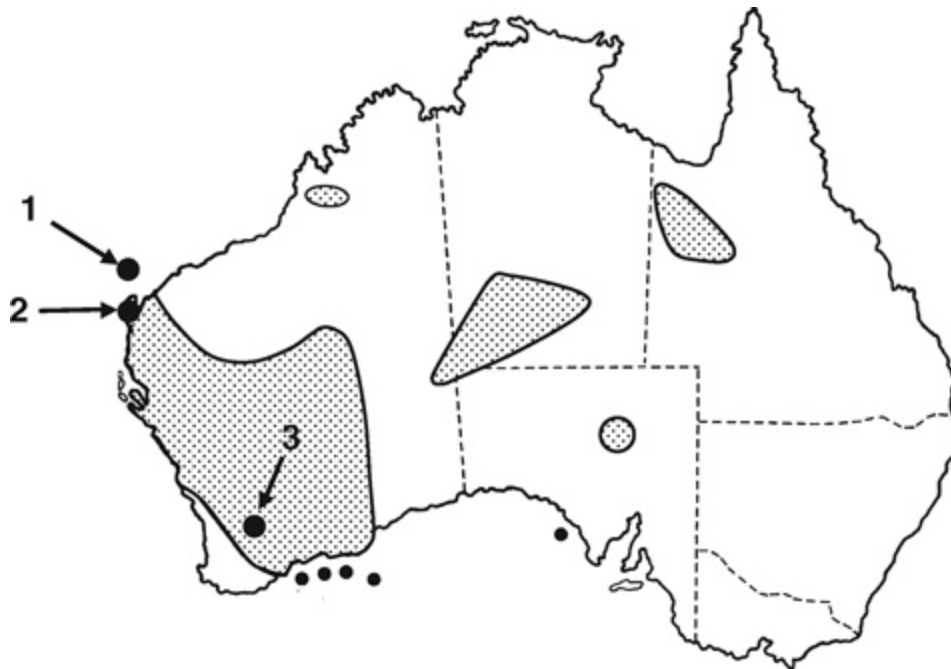
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**Box 2.3 Island populations of black-footed rock wallabies have persisted for 1600 or more generations at small sizes, are highly inbred, have low levels of genetic diversity and exhibit inbreeding depression (Eldridge *et al.* [1999](#)).**

Rock wallabies are 1m tall marsupials inhabiting rocky outcrops on the Australian mainland and on offshore islands (see map below). The Barrow Island population of black-footed rock wallabies (location 1) has been isolated from the mainland for 8000 years (about 1600 generations) and has a relatively small population size. Genetic diversity on Barrow Island and in other island populations (dots without numbers) is markedly lower than in mainland sites at Exmouth (2) and Wheatbelt (3).



## Black-footed rock wallaby



Population (location)	Proportion of loci polymorphic	Mean no. alleles/locus	Average heterozygosity
Barrow Island (1)	0.1	1.2	0.05
Mainland			
Exmouth (2)	1.0	3.4	0.62
Wheatbelt (3)	1.0	4.4	0.56

Since its isolation, the Barrow Island population has obviously survived stochastic fluctuations and catastrophes. It has an inbreeding coefficient of 0.91 and displays inbreeding depression compared to the mainland population. The frequency of lactating females is 92% in mainland rock wallabies, but only 52% on Barrow Island. Island populations have been viewed as ideal sources for restocking depleted or extinct mainland populations, especially in Australasian species. However, they are poor candidates for translocations, especially if alternative mainland populations exist, as they often have low genetic diversity and are inbred.

## Computer simulations predict that inbreeding increases extinctions

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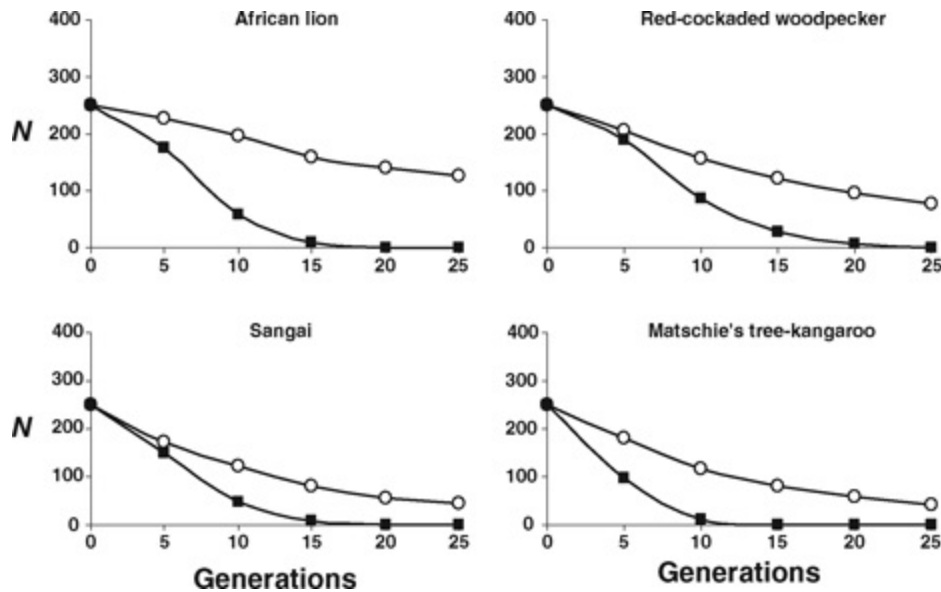
Computer projections show that inbreeding elevates extinction risk for most outbreeding wild populations

Computer projections incorporating factual life history information are often used to assess the combined impact of all deterministic and stochastic factors on the probability of extinction of populations (see [Chapter 22](#)). Population size, births and survival rates and their variation over age and through time, together with measures of inbreeding depression, changes in habitat quality, etc. form the input. Stochastic models are then run through repeated cycles to project the fate of many replicate populations into the future.



Almost all computer projections, using a range of outbreeding bird, mammal and reptile life cycles yielded substantial increases in extinction risk when the effects of inbreeding were included, as compared to runs where they were excluded (O'Grady *et al.* [2006](#); Schiegg *et al.* [2006](#)). Results for four different species are shown in [Fig. 2.4](#). The differences in population

size trajectories translate into increases in extinction risk over time. Inbreeding depression in the wild reduced median times to extinction by 30–40% for populations initiated with 50–1000 individuals.



**Fig. 2.4** Impact of inbreeding on population size. Computer projections of population sizes for threatened wild populations of three mammal and one bird species, when the deleterious effects of inbreeding are included (■), or excluded (○) (O'Grady *et al.* 2006).

## Most species are not driven to extinction before genetic factors impact them

An experimental test across a broad range of taxa refuted the claim that most species are driven to extinction before genetic factors can impact them

Several authors have suggested that species will go extinct before being



impacted by genetic factors (Lande [1988](#); but see Frankham [2005](#)). However, a comprehensive meta-analysis involving 170 paired comparisons revealed that the majority of threatened taxa (77%) exhibited reduced genetic diversity (Spielman *et al.* [2004a](#)) ([Box 2.4](#)), leading the authors to reject the ‘no genetic impact’ scenario for most taxa. This effect was consistent across a broad array of major taxa. The authors were not able to determine what caused the taxa to become threatened. However, the results indicate that most threatened taxa are now suffering a reduced ability to evolve (median reduction in heterozygosity of 40%), elevated inbreeding and reduced reproductive fitness, all of which indicate that they will suffer elevated extinction risk into the future. Further, there is still time for genetic factors to impact taxa with normal levels of genetic diversity. For example, vulnerable taxa that form the majority of the data set have a probability of extinction of only approximately 10% within 100 years (IUCN [2007](#)).

## Relationship between loss of genetic diversity and extinction

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To cope with ever-changing environments, species need genetic diversity to evolve, or face extinction

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Natural populations face continuous pressures from environmental changes, including new infectious diseases, pests, parasites, food sources, competitors, predators, pollution and global climate change. Naturally outbreeding species with large populations normally possess large genetic differences among individuals, allowing adaptations to such pressures ([Chapters 3 and 6](#)). Evolutionary responses to environmental change have been observed in many species ([Chapter 6](#)). For example, over 200 species of moths have evolved black body colours (melanics) to aid in camouflage in response to industrial pollution (Kettlewell [1973](#)).

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Genetic diversity is lost in small populations

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Small populations typically have lower levels of genetic diversity than large populations. This is because alleles are lost in small populations during the random sampling as parents pass their genes to their offspring (termed **genetic drift**). Loss is inversely proportional to population size ([Chapters 8 and 11](#)).

---

Populations with lower genetic diversity are poorer at coping with environmental extremes and infectious diseases than populations with higher genetic diversity

---

Genetic variation allows populations to tolerate a wider range of environmental regimes (Bijlsma & Loeschcke [2005](#)). These include ability to tolerate climatic extremes, heavy metal pollutants, herbicides, pesticides, etc. Humans are imposing increasing rates of such environmental change. If populations are to cope with these changes, they require genetic diversity.

Loss of genetic diversity is predicted to reduce the ability of populations to evolve and increases their extinction risk. However, it is difficult to delineate where extinctions of natural populations have been caused by lack of genetic variation, since multiple factors interact in the extinction vortex.

## Loss of genetic diversity at self-incompatibility loci causes extinctions in plants

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Loss of self-incompatibility alleles in small populations of many plant species leads to reduced reproductive fitness

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The most direct evidence of a relationship between loss of genetic diversity and increased risk of extinction comes from studies of **self-incompatibility** loci in plants. About half of all flowering plant species have genetic systems that reduce or prevent self-fertilization (Richards [1997](#)). Self-incompatibility is regulated by a cluster of loci that typically have many alleles within a species (Castric & Vekemans [2004](#)). If the same allele is present in a pollen grain and the stigma, fertilization by that pollen grain will not occur. Self-incompatibility is presumed to have evolved to avoid the deleterious effects of inbreeding.

Self-incompatibility alleles are lost by genetic drift in small populations, leading to a reduction in the proportion of pollen that can fertilize the eggs of any individual and eventually to reduced seed set and extinction ([Chapters 9 and 17](#)). For example, the Lakeside daisy population from Illinois declined to three plants and had so few self-incompatibility alleles left that it did not reproduce for 15 years – it was functionally extinct (Demauro [1993](#)). Plants did however produce viable seed when fertilized with pollen from large populations in Ohio or Canada. The endangered grassland daisy in eastern Australia also exhibits reduced fitness in smaller populations with reduced numbers of self-incompatibility alleles (Pickup & Young [2007](#)). While reduced fitness due to loss of self-incompatibility alleles has only been documented in a handful of plant species (Castric & Vekemans [2004](#)), it is predicted to become a problem in most threatened, self-incompatible plants.



Endangered grassland daisy

A similar effect of reduced fitness due to loss of genetic diversity occurs at the sex locus in haplo-diploid species (see [Chapter 17](#)).

### **Loss of genetic diversity decreases resistance to diseases, pests and parasites**

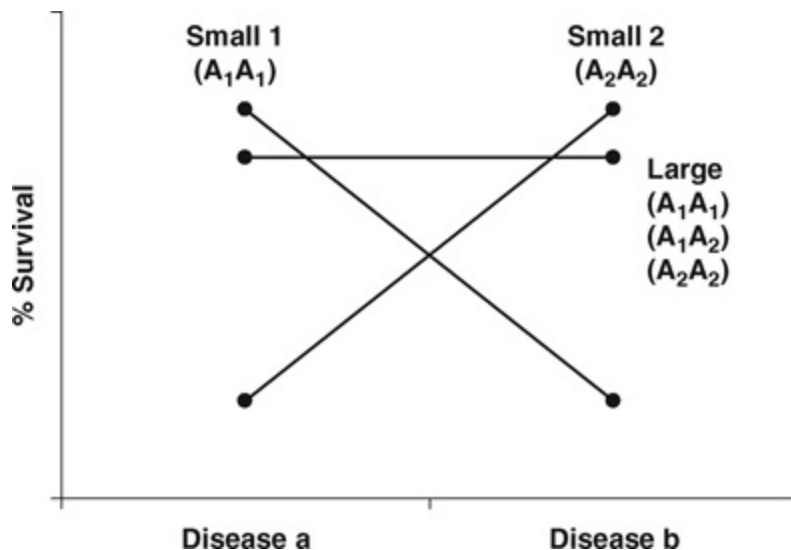
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Populations with reduced genetic diversity are expected to suffer more seriously from pests, parasites and infectious diseases than those with high genetic diversity

---

Novel pathogens constitute one of the most serious threats to all species. For example, a wide range of bird and mammal species are susceptible to the newly emerged H5N1 influenza strain, whilst many species of amphibians are susceptible to the chytrid fungus (Robertson *et al.* [2006](#)). Loss of genetic

diversity severely diminishes the capacity of populations to respond to pests, parasites and infectious diseases ([Chapters 6, 8 and 11](#)). Following sequential assaults by different pathogens, populations with high genetic diversity are more likely to persist than populations with low genetic diversity (Penn *et al.* 2002) ([Fig. 2.5](#)). Negative associations between low genetic diversity and pathogen resistance have been found in deer mice, Soay sheep, birds, topminnow fish, bumblebees, ants and fruit flies (Hughes & Boomsma 2004; Spielman *et al.* 2004b; Whiteman *et al.* 2006).



**Fig. 2.5** Hypothetical example of the relationship between genetic diversity and disease resistance. Two small inbred populations, each homozygous for different alleles, are resistant to one pathogen, but not to the other. Conversely, a larger population containing both alleles can resist both pathogens.

The **major histocompatibility complex** (MHC) in vertebrates is a large cluster of loci involved in recognizing pathogen antigen molecules and regulating immune responses. The more heterozygous an individual is, the more pathogens the individual can respond against. Genetic diversity at these loci is amongst the highest known for any loci, and is maintained by selection favouring heterozygotes and by selection favouring rarer alleles ([Chapter 9](#)). Despite this selection, alleles are lost by genetic drift in small populations, increasing the chance that a pathogen that can kill one individual can kill all (Sommer 2005). For example, higher heterozygosity at three components of

the MHC is associated with longer survival following HIV infection in humans (Carrington *et al.* 1999). Tasmanian devils in Australia are in rapid decline due to a clonal facial tumour that is spread by biting. The devils have such low MHC genetic diversity they do not identify the clonal tumour as foreign tissue and fail to reject it (Siddle *et al.* 2007).



Tasmanian devil

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### **Box 2.4 Meta-analyses (Arnquist & Wooster 1995; Lipsey & Wilson 2001; Møller & Jennions 2001; MetaWin 2007)**

We include this box as meta-analyses are not a regular part of the statistical training of most biologists, yet they have become an extremely important tool, especially in conservation and evolutionary biology.

In many areas of conservation biology there are conflicting reports, as individual studies are typically small. An overview is frequently obtained by combining all the available published and unpublished data into a single analysis (a meta-analysis). For example, there have already been six meta-analyses referred to in this chapter, three on impacts of inbreeding in captive and wild animals and plants (Ralls & Ballou 1983; Ralls *et al.* 1988; Crnokrak & Roff 1999), two on island populations (Frankham 1997, 1998) and one comparing genetic diversity in threatened and taxonomically related non-threatened taxa (Spielman *et al.* 2004a). Throughout this book we refer to many other meta-analyses, including those on correlations between heterozygosity and quantitative genetic variation (Chapter 5), the relationship between genetic diversity and population size (Chapter 11), the magnitude of effective to census

size ratios ([Chapter 11](#)), inbreeding depression in benign and stressful environments ([Chapter 13](#)), comparative levels of genetic diversity in invasive species in introduced and source locations ([Chapter 18](#)) and minimum viable population sizes ([Chapter 22](#)).

There are frequently problems of **statistical power** in conservation biology, as many studies have involved only small samples. For example, a high correlation of 0.75 between genetic diversity and population size was non-significant in a plant species, as only four populations were studied (Frankham [1996](#)). It requires a correlation of 0.95 to be significant with only four pairs of observations. This issue can be addressed by meta-analyses, where overall analyses have much larger combined sample sizes, and thus higher statistical power. Further, meta-analyses may combine data across many species.

A range of methods are used in the combined analyses, including:

- vote counting
- combined probability analyses
- analyses of effect size.

The vote counting method simply assesses the proportion of differences that are in the predicted direction. For example, 41 of 44 mammal populations showed higher juvenile mortality in inbred compared to outbred progeny (Ralls & Ballou [1983](#)). This is wildly different from the null expectation of equal numbers in each category. This method has relatively low statistical power and does not weight the different data sets by their sample sizes.

The combined probability method simply computes an overall probability from the probabilities reported by different studies, populations or species. For example, in the study referred to in [Chapter 11](#) the regressions of proportions of initial microsatellite heterozygosity retained on inbreeding coefficient for loci on three different fruit fly chromosomes were  $-1.142 \pm 0.115$  ( $P = 0.114$ ),  $-1.216 \pm 0.071$  ( $P = 0.003$ ) and  $-1.040 \pm 0.097$  ( $P = 0.342$ ), respectively. The probabilities all measure the deviation from the theoretical prediction of a slope of  $-1$ . Using Fisher's Combined Probability Test  $\chi^2 = -2 \sum \ln P$ , with 2 degrees of freedom for each  $P$  value, yielded

$$\chi^2 = 4.34 + 11.62 + 2.14 = 18.1$$

with  $df = 6$ , and  $P = 0.006^{**}$ .

Thus, heterozygosity is lost at a significantly faster rate than predicted by theory.

Until recently, Fisher's Combined Probability Test was used to combine probabilities. However, Whitlock (2005) has shown that the weighted Z-method has greater power and precision than Fisher's method. In this method, each of the individual probabilities is converted into standard normal deviates and the overall Z value computed as:

$$Z_s = \frac{\sum Z_i w_i}{\sqrt{\sum w_i^2}}$$

where  $w_i$  are the weights for each individual study and the values are summed over all the studies. Weights indicate the precision of individual estimates and may be the inverse of the standard errors squared, sample sizes, etc. For the example above, the three Z values are 1.20, 2.75 and 0.41, with weights  $1/(\text{standard error})^2$  of 76.1, 197.2 and 105.6 yielding

$$\text{weighted } Z = \frac{\sum Z w}{\sqrt{\sum w^2}} = 2.86$$

The probability is determined by reference to normal distribution tables or software, yielding  $P = 0.002^{**}$ . This is clearly an overall highly significant signal. Combined probability methods can be used even when diverse statistical methods ( $t$ ,  $z$ ,  $F$  or chi-square) have been used in different studies.

Analyses of effect size can be used to determine the magnitude of the overall effect from the weighted mean. Here, individual experiments are weighted according to sample size, or degrees of freedom, or sampling variation. In the case of the correlations between heterozygosity and quantitative genetic variation (Chapter 5), the weighted mean correlation was  $0.22^* \pm 0.05$  (Reed & Frankham 2001). This should give the most reliable measure of the magnitude of the effect. Meta-analyses may also allow tests of effects that have not been addressed in any individual study.

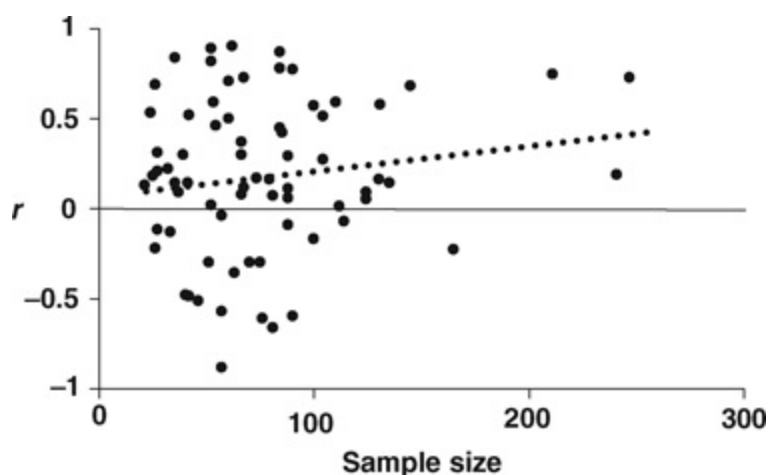


For example, in the above-mentioned study, a test was conducted to determine whether correlations between heterozygosity and quantitative genetic variation differed between life history (fitness) characters and morphology (peripheral to fitness). The two differed significantly with the former showing a correlation not differing from zero and the latter a significant but modest positive correlation.

The main reservation about meta-analyses is publication bias, also referred to as the 'file drawer problem'. Do publications predominantly include only significant results, leading to a bias in the magnitude of differences? This bias exists in all reviews of the literature, but in the case of meta-analyses it is recognized and explicitly tested for. Three common methods to check for bias are:

- funnel plots of effect size versus sample size
- regression of effect size on sample size, and
- computing the number of unpublished studies in the non-expected direction required to remove the significance of the effect.

If there is no bias we expect the plot of effect size versus sample size to approximate to a funnel, with the variation among estimates decreasing as sample size increases (see figure below). A publication bias typically leads to a deficiency of small studies with low effect sizes.



A related test is to regress effect size on sample size. A non-significant regression indicates no publication bias, whilst a significant negative regression indicates publication bias. Regression of correlations between heterozygosity and quantitative genetic variation from Reed & Frankham

(2001) on sample size (figure above) gave  $b = 0.0014 \pm 0.0011$  ( $P = 0.22^{\text{ns}}$ ,  $r^2 = 0.022$ ), a non-significant regression, indicating lack of publication bias.

Another indicator for publication bias is to ask how many times differences in the ‘wrong’ direction have to be added to the data to make it non-significant. In the data set of Spielman *et al.* (2004a) on comparative levels of heterozygosity in threatened and related non-threatened taxa (Chapter 3), the mean of the 38 taxa with positive differences (in opposite direction to the hypothesis) had to be added to the real data 127 times before the difference became non-significant. Thus, the study was robust.

The MetaWin software package is designed for meta-analyses.

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## Summary

1. Inbreeding and loss of genetic diversity are of conservation concern as they increase the risk of extinction.
2. Inbreeding reduces reproductive fitness in essentially all well-studied populations of naturally outbreeding species.
3. Inbreeding increases the risk of extinction in captive populations, and there is now substantial evidence that it is one of the factors causing extinctions of wild populations.
4. Loss of genetic diversity reduces the ability of species to evolve to cope with environmental change, thus also contributing to elevated extinction risk in small populations.

## Further reading

Frankham (2005) Review on the role of genetics factors in extinctions.

Keller & Waller (2002) Review on inbreeding effects in wild populations.

Lande (1988) Interpreted as saying that demographic and environmental

factors are likely to drive populations to extinction before genetic factors become important.

Lipsey & Wilson (2001) *Practical Meta-Analysis*. Relatively easy-to-follow textbook on meta-analysis.

O'Grady *et al.* (2006) Realistic computer projections showed that inbreeding depression substantially reduces median times to extinction for most threatened outbreeding species.

Spielman *et al.* (2004a) Tested the presumption that most species are driven to extinction before genetic factors impact them and rejected it for most threatened species.

Young *et al.* (2000) A review of studies on the deleterious impacts of loss of self-incompatibility alleles on an endangered grassland daisy in Australia.

## Software

METAWIN: Commercial software for meta-analyses.  
[www.metawinsoft.com/](http://www.metawinsoft.com/)

VORTEX: Free software for computer projection of the fate of populations due to deterministic and stochastic factors including inbreeding (Lacy *et al.* 2005). [www.vortex9.org/](http://www.vortex9.org/)

## Problems

- 2.1 Inbreeding. What is inbreeding?
- 2.2 Inbreeding. Why is inbreeding of conservation concern?
- 2.3 Relationship between population size and extinction. What factors could account for the association of extinction rates and population size in bighorn sheep ([Fig. 2.3](#))?
- 2.4 Reproductive fitness. What is reproductive fitness? Name at least four components of a species' life history that contribute to its reproductive fitness.

## **Practical exercises: Computer projections**

Use the VORTEX simulation package to determine the extinction risk of the following populations when the effects of inbreeding on reproductive fitness are included and when they are ignored. Use the following input data for Mauritius pink pigeon and the Capricorn silvereye, plus that for the golden lion tamarin ([Table 22.1](#)). Numbers of individuals in each species are adjusted for these exercises. Look for other data files on the book's website (see Preface) and the VORTEX website.

### **Pink Pigeon Vortex Input Data**

This provides input data for the scenario WITH INBREEDING. Create another scenario WITHOUT INBREEDING by copying the “Inbreeding” scenario and deselecting the inbreeding options.

Tab	Vortex parameter	User input
Scenario Settings	Project Name	Pink Pigeon
	Scenario Name	Inbreeding
	Number Iterations	1000
	Number Years	100
	Definition of Extinction	Only one sex
	Populations	1
Species Description	Inbreeding Depression?	yes
	Lethal Equivalents	3.14
	Percent due to Recessive Lethals	50
	EV Concordance Repro and Survival?	yes
	Number of Types of Catastrophes	3
Labels and State Vars.	Ignore	
Dispersal	Ignore	
Reproductive System	Mating System	Monogamous
	Age First Offspring to Females	1
	Age First Offspring to Males	1
	Maximum Age Reproduction	15
	Maximum Number Progeny/year	2
	Sex Ratio at Birth	55
	Density Dependent Reproduction?	N
Reproductive Rates	% Adult Females Breeding	40.0
	Number of Offspring/F/year	Specify exact distribution
	Data: % Females producing 1 Offspring	87.5
	Data: % Females producing 2 Offspring	12.5 (automatic)
Mortality Rates	<b>Female</b> Mortality from age 0 to 1	25.0
	SD in 0 to 1 due to EV	5.0
	Annual Mortality after age 1	15.0
	SD After Age 1	2.0
	<b>Male</b> Mortality from age 0 to 1	25.0
	SD in 0 to 1 due to EV	5.0
	Annual Mortality after age 1	15.0
	SD After Age 1	2.0
	Labels	Ignore
	<b>Catastrophe 1</b>	
Catastrophes	Global/Local	Ignore
	Frequency %	6.0
	Severity-Reproduction	1.0
	Severity-Survival	0.5
	<b>Catastrophe 2</b>	
	Global/Local	Ignore
	Frequency %	3.0
	Severity-Reproduction	1.0
	Severity-Survival	0.9

Tab	Vortex parameter	User input
	<b>Catastrophe 3</b>	
	Global/Local	<i>Ignore</i>
	Frequency %	1.0
	Severity–Reproduction	0.9
	Severity–Survival	0.5
Mate Monopolization	% Males in the Breeding Pool	80.0
Initial Population Size	Start with	Stable Age Distribution
	Initial Population Size	16
Carrying Capacity	K	35
	SD in K due to EV	0.0
	Future change in K?	No
Harvest	Harvest?	No
Supplementation	Supplement?	No
Genetic Management	<i>Ignore</i>	No

## Silver Eye Vortex Input Data

This provides input data for the scenario WITH INBREEDING. Create another scenario WITHOUT INBREEDING by copying the “Inbreeding” scenario (see program's help file) and deselecting the inbreeding options.

Tab	Vortex parameter	User input
Scenario Settings	Project Name	Silver Eye
	Scenario Name	Inbreeding
	Number Iterations	1000
	Number Years	100
	Definition of Extinction	Only one sex
	Populations	1
Species Description	Inbreeding Depression?	yes
	Lethal Equivalents	3.14
	Percent due to Recessive Lethals	50
	EV Concordance Repro and Survival?	yes
	Number of Types of Catastrophes	1
Labels and State Vars.	<i>Ignore</i>	
Dispersal	<i>Ignore</i>	
Reproductive System	Mating System	Monogamous
	Age First Offspring to Females	1
	Age First Offspring to Males	1
	Maximum Age Reproduction	11
	Maximum Number Progeny/year	5
	Sex Ratio at Birth	50
	Density Dependent Reproduction?	N

Tab	Vortex parameter	User input
Reproductive Rates	% Adult Females Breeding	40.0
	Number of Offspring/F/year	Normal Distribution
	Mean	1.927
	Standard Deviation	1.245
Mortality Rates	<b>Female</b> Mortality from age 0 to 1	42.3
	SD in 0 to 1 due to EV	8.93
	Annual Mortality after age 1	37.6
	SD after age 1	9.31
	<b>Male</b> Mortality from age 0 to 1	42.3
	SD in 0 to 1 due to EV	9.31
	Annual Mortality after age 1	37.6
	SD after age 1	9.31
Catastrophes	Labels	<i>Ignore</i>
	<b>Catastrophe 1</b>	
	Global/Local	<i>Ignore</i>
	Frequency %	10.0
	Severity–Reproduction	1.0
	Severity–Survival	0.645
Mate Monopolization	% Males in the Breeding Pool	100
Initial Population Size	Start with	Stable Age
		Distribution
Carrying Capacity	Initial Population Size	342
	K	500
	SD in K due to EV	50.0
	Future change in K?	No
Harvest	Harvest?	No
Supplementation	Supplement?	No
Genetic Management	<i>Ignore</i>	No



# **Section I Evolutionary genetics of natural populations**

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Since our objective in conservation genetics is to preserve species as dynamic entities capable of evolving to adapt with environmental change, it is essential to understand the natural forces determining evolutionary change. Such information is indispensable if we are to understand how to genetically manage threatened and endangered populations. Since evolution at its most basic level is a change in the genetic composition of a population, it only occurs when there is genetic diversity. Consequently, we need to appreciate how genetic diversity arises, how it is lost, how much genetic diversity exists in populations, what forms of genetic diversity exist and how it is measured.

## **Extent of genetic diversity**

**Chapter 3** introduces methods for measuring genetic diversity for DNA, proteins, deleterious alleles and quantitative characters, and documents levels of genetic diversity for them. Most large populations of animals and plants contain extensive genetic diversity. However, levels of genetic diversity are often reduced in small populations, island populations and endangered species.

## **Genetic constitution of populations**

To evaluate changes in genetic diversity, we must have means for quantifying

it. **Chapter 4** covers the estimation of allele (gene) frequencies and heterozygosity that are used to describe diversity at single loci. **Chapter 5** describes the measures used to characterise genetic diversity for quantitative characters, especially the concept of heritability. Quantitative characters are centrally involved in the major areas of conservation concern, the ability to genetically adapt to environmental change (evolutionary potential), the deleterious effects of inbreeding (inbreeding depression) and the deleterious effects that sometimes occur when different populations are mixed (outbreeding depression).

## Changes in genetic diversity

Genetic diversity is generated by mutation, and the frequencies of different alleles change due to migration, selection and chance. The roles of these forces in large populations are discussed in **Chapters 6–9**. Since environmental change is ubiquitous, species must adapt through natural selection to better cope with these changes (**Chapter 6**). Deleterious mutations exist in populations due to a balance between input of deleterious mutations and their removal by natural selection (**Chapter 7**).

**Chapter 8** is concerned with evolution in small populations, the situation for most species of conservation concern. Evolutionary processes in small populations differ in two critical ways from those in large populations:

- chance has a much greater role, and
- selection has a lesser impact.

**Chapter 9** considers how genetic diversity is maintained in natural populations. This information provides the background for devising genetic management procedures to retain genetic diversity and evolutionary potential in threatened species. The relative importance of chance, mutation and selection in maintaining genetic diversity vary among different traits (DNA, proteins, visible polymorphisms, gene clusters and quantitative characters). A proportion of the diversity in populations is selectively neutral. It arises by

mutation and its fate is influenced by chance sampling events in finite populations. Other alleles are actively maintained by the action of balancing selection. A substantial proportion of the genetic diversity for fitness characters is in mutation–selection balance. Even alleles subject to selection are lost by chance in small populations. Our understanding of evolution has been enhanced by recent sequence analysis of genomes and by multilocus microarray studies on gene expression. **Chapter 10** on population genomics describes new and enhanced insights about evolutionary processes and discusses how they might contribute to conservation.

## Chapter 3 Genetic diversity

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Genetic diversity is required for populations to adapt to environmental change. Large populations of naturally outbreeding species typically have extensive genetic diversity, but it is usually reduced in small populations and species of conservation concern

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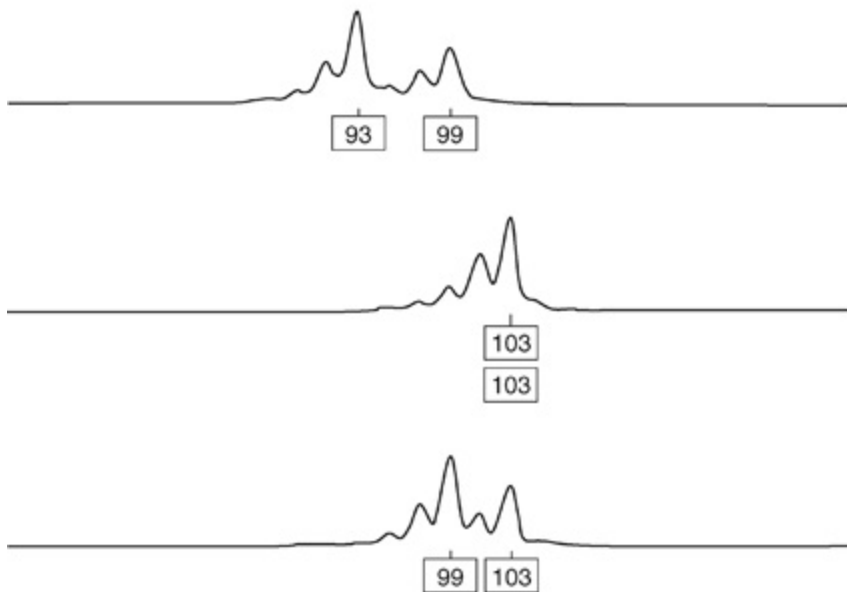
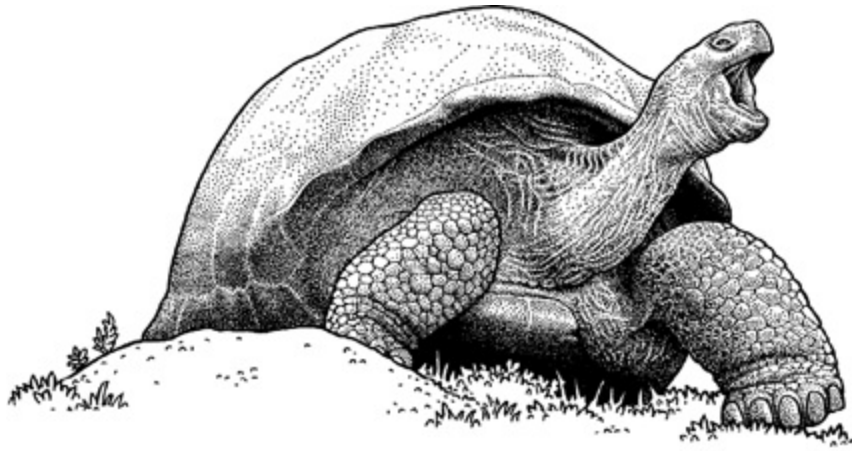
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### Terms

Allelic diversity, allozyme, amplified fragment length polymorphism (AFLP), autoradiograph, chloroplast DNA (cpDNA), DNA fingerprint, electrophoresis, exon, genetic distance, genetic load, genome, haplotype, heterozygosity, intron, inversion, locus, microsatellite, mitochondrial DNA (mtDNA), monomorphic, polymerase chain reaction (PCR), polymorphic, quantitative character, quantitative genetic variation, randomly amplified polymorphic DNA (RAPD), restriction enzyme, restriction fragment length polymorphism (RFLP), silent substitution, single nucleotide polymorphism (SNP), synonymous substitution

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A Galápagos tortoise and output from a DNA sequencing machine illustrating genetic diversity among individuals at a microsatellite locus in this species

## Importance of genetic diversity

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Loss of genetic diversity in small populations reduces the ability of populations to evolve with environmental change. It is usually associated with reduced reproductive fitness in species that naturally outbreed

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IUCN recognizes the need to conserve genetic diversity for three reasons (McNeely *et al.* [1990](#)). First, genetic diversity is needed for populations to evolve to adapt to environmental change. Second, loss of genetic diversity is usually associated with inbreeding and reduction in reproduction and survival in species that normally outbreed. Third, genetic diversity contributes to ecosystem diversity. For example, plant genetic diversity relates to species diversity of phytophagous species and genetic diversity makes ecosystems more resilient to environmental shocks (Reusch *et al.* [2005](#); Crutsinger *et al.* [2006](#)).

This chapter addresses the basis of conservation concerns about genetic diversity, defines what it is, describes methods for measuring it, and reviews the evidence on its extent in threatened and non-threatened species.

## Genetic diversity enables evolutionary change

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Genetic diversity is the raw material for adaptive evolutionary change

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Species face ever-changing environments, be they climatic changes, pollution or the introduction of novel competitors, diseases, pests or parasites ([Chapter 6](#)). Species must evolve to cope with these changes, or become extinct. Genetic diversity in a population reflects its evolutionary potential. Short-term evolution cannot occur in populations lacking genetic diversity, while populations with extensive genetic diversity can evolve relatively rapidly in response to environmental change. For example, plants with genetic diversity for heavy-metal tolerance (*Agrostis tenuis* and bunch grass) were able to colonize soils polluted with copper, zinc and cadmium on mine wastes in Wales, UK by evolving tolerant forms. Plant species without the appropriate genetic diversity failed to colonize (Bradshaw [1991](#)). Similarly, the American chestnut was driven almost to extinction by an introduced disease to which it had no genetic diversity for resistance. Industrial melanism has evolved in about 200 genetically diverse species of moths in areas subject to industrial pollution (Kettlewell [1973](#)). Many ‘pest’ species have evolved resistance to insecticides, herbicides, antibiotics and other biocontrol agents (Georghiou [1986](#); McKenzie [1996](#)).

## Relationship between genetic diversity and reproductive fitness

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Loss of genetic diversity is related to reduction in reproductive fitness in naturally outbreeding species

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Reduction in genetic diversity in random mating species is directly related to inbreeding, and thus to reduced reproductive fitness ([Chapters 2 and 11–13](#)). As expected, there is a positive correlation between genetic diversity and average fitness across populations (Reed & Frankham [2003](#); Leimu *et al.* [2006](#)).

---

Maintenance of genetic diversity is a primary objective in the management of wild and captive populations of threatened species

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Captive breeding and wildlife management programs typically recognise the importance of minimizing inbreeding and loss of genetic diversity. Management actions include consulting pedigrees when establishing matings or choosing individuals to reintroduce into the wild ([Chapters 19](#) and [20](#)). Levels of genetic diversity can be analysed and monitored in wild populations of endangered species, and gene flow between isolated wild populations may be augmented ([Chapter 17](#)).

## What is genetic diversity?

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Genetic diversity is the variety of alleles and genotypes present in the group under study (population, species or group of species)

---

Genetic diversity is manifested by differences in many characters, including eye, skin and hair colour in humans, colour and banding patterns of snail shells, flower colours in plants, and in the proteins, enzymes and DNA sequences of almost all organisms. For example, the vast variety of dog breeds reflect genetic diversity in this species (Vila *et al.* [1997](#)). Selection based on phenotypes has produced breeds of different size (St Bernard versus chihuahua), behaviour (guard dogs, hunting dogs, sheep dogs, cattle dogs,



etc.), shape (bulldogs, dachshunds), etc., as illustrated below ([Fig. 3.4](#)).

Genes are sequences of nucleotides in a particular segment (locus) of a DNA molecule. Genetic diversity represents slightly different sequences. In turn, DNA sequence variants may result in amino acid sequence differences in the protein coded for by the locus. Such protein variation may result in functional biochemical or morphological dissimilarities that cause differences in reproductive rate, survival or behaviour of individuals. Similarly, genetic diversity among loci that program the development of individuals can affect the final phenotype.

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Genetic diversity in populations is generated by mutations or introduced by migration

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All genetic diversity is originally generated by mutations that change the nucleotides in a sequence of DNA ([Chapter 7](#)). Genetic diversity may also be introduced into populations by immigrants.

Terminology used to describe genetic diversity is defined in [Table 3.1](#). Genetic diversity is typically described using **polymorphism**, average **heterozygosity** and **allelic diversity**. For example, in African lions 23% of protein-coding loci were variable (polymorphic), 7.1% of loci were heterozygous in an average individual, and there was an average of 1.27 alleles per locus (allelic diversity), as assessed by allozyme electrophoresis (see Example 3.1). These levels of genetic diversity are typical of allozyme variation for non-threatened mammals. By contrast, endangered Asiatic lions have low genetic diversity ([Box 3.1](#)). Further details on characterization of

genetic diversity are given in [Chapter 4](#).

**Table 3.1** *Terminology used to describe genetic diversity*

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**Locus** (plural **loci**) The site on a chromosome at which a particular gene is located, or a segment of DNA or an individual gene, e.g. the segment of DNA coding for the alcohol dehydrogenase enzyme is a separate locus from those coding for haemoglobins. Molecular loci, such as microsatellites (see below), are simply segments of DNA that may have no functional products.

**Alleles** Different variants of the nucleotide sequence at the same locus (gene) on homologous chromosomes, typically labelled as  $A_1, A_2, A_3, A_4$ , for the four different alleles of the A locus.

**Genotype** The combination of alleles present at a locus in an individual, e.g.  $A_1A_1, A_1A_2$  or  $A_2A_2$ .

**Genome** The complete genetic material of a species, or individual. The entire DNA nucleotide sequence, including all of the loci and regions between them and all of the chromosomes.

**Homozygote** An individual with two copies of the same allele at a locus, e.g.  $A_1A_1$ .

**Heterozygote** An individual with two different alleles at a locus, e.g.  $A_1A_2$ .

**Allele frequency** The relative frequency of a particular allele in a population (often referred to as gene frequency). For example, if a population of a diploid species has 2  $A_1A_2$  individuals and 8  $A_2A_2$  individuals, then there are 2 copies of the  $A_1$  allele and 18 of the  $A_2$  allele. Thus, the  $A_1$  allele has a frequency of 0.1 and the  $A_2$  allele a frequency of 0.9.

**Polymorphic** The presence in a species of two or more alleles at a locus, e.g.  $A_1$  and  $A_2$ . Polymorphic loci are usually defined as having the most frequent allele at a frequency of less than 0.99, or less than 0.95 (to minimize problems with different sample sizes).

**Monomorphic** A locus in a population is monomorphic if it has only one allele present, e.g.  $A_1$ . All individuals are homozygous for the same allele.

**Proportion of loci polymorphic (P)** Number of polymorphic loci / total number of loci sampled. For example, if 3 of 10 sampled loci are polymorphic, and 7 are monomorphic,

$$P = \frac{3}{10} = 0.3.$$

**Average heterozygosity (H)** Average proportion of loci that is heterozygous. For example, if the proportions of individuals heterozygous at 10 loci in a population are 0.2, 0.4, 0.1, 0, 0, 0, 0, 0, 0, and 0, then

$$H = \left( \frac{0.2 + 0.4 + 0.1 + 0 + 0 + 0 + 0 + 0 + 0 + 0}{10} \right) = 0.07.$$

This is the **observed heterozygosity**. Typically, expected heterozygosities (Chapter 4) are reported, as they are less sensitive to sample size than observed heterozygosities, but the two are usually numerically similar in random mating populations.

**Allelic diversity (A)** Average number of alleles per locus

For example, if the number of alleles at 10 loci are 2, 3, 2, 1, 1, 1, 1, 1, 1 and 1, then

$$A = \left( \frac{2 + 3 + 2 + 1 + 1 + 1 + 1 + 1 + 1 + 1}{10} \right) = 1.4.$$

**Co-dominance** Situation where all genotypes can be identified from phenotypes, i.e.  $A_1A_1, A_1A_2$ , and  $A_2A_2$  can be distinguished. This contrasts with dominance where the phenotypes of some genotypes are indistinguishable.

**Genetic distance** A measure of the genetic difference between allele frequencies in two populations or species, e.g. Nei's genetic distance (Chapter 16). These are usually based on many loci.

---

### Example 3.1 Genetic diversity in African lions (Newman et al. 1985)

A survey of protein electrophoretic variation at 26 loci in African lions revealed 20 with no variation (monomorphic), and six loci with variation (polymorphic). Frequencies of alleles at the polymorphic loci are shown, along with the proportions of individuals in the population that were heterozygous ( $H$ ) for each of the six loci.

Enzyme locus	Allele			Heterozygosity ( $H$ )
	1	2	3	
ADA	0.56	0.33	0.11	0.564
DIAB	0.61	0.39		0.476
ESI	0.88	0.12		0.211
GPI	0.85	0.15		0.255
GPT	0.89	0.11		0.196
MPI	0.92	0.08		0.147
20 other loci monomorphic	1.00			0

Since six of 26 loci were variable, the proportion of loci polymorphic ( $P$ ) is

$$P = \frac{6}{26} = 0.23$$

Thus, 23% of loci are estimated to be polymorphic in African lions.

The proportion of loci heterozygous in an average individual ( $H$ ) is computed as follows:

$$H = \frac{[0.564 + 0.476 + 0.211 + 0.255 + 0.196 + 0.147 + (20 \times 0)]}{26} = 0.071$$

Thus, 7.1% of loci are heterozygous in an average lion.

The allelic diversity ( $A$ ) is computed as follows:

$$A = \frac{[3 + (5 \times 2) + (20 \times 1)]}{26} = 1.27 \text{ alleles per locus}$$

Thus, there is an average of 1.27 alleles per locus.

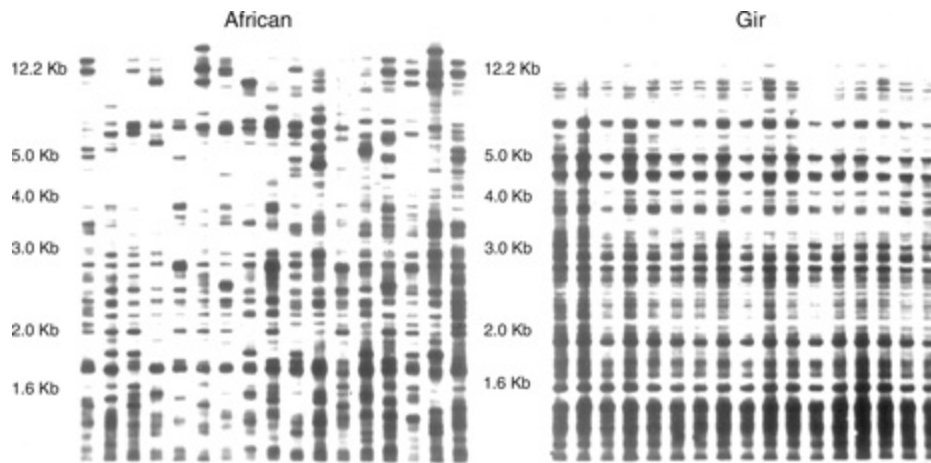
### Box 3.1 Very low genetic diversity in endangered Asiatic lions from Gir Forest, India (O'Brien 1994)



Asiatic lion

Wild Asiatic lions occur only in the Gir Forest of northwest India, in a relict group of fewer than 250 individuals. O'Brien and co-workers measured genetic diversity for 50 allozyme loci, and for DNA fingerprints (see figure below from Gilbert *et al.* 1991). Levels of genetic diversity for Gir lions and for several populations of non-endangered African lions with much larger population sizes are given below.

	Allozymes		DNA fingerprints
	<i>P</i>	<i>H</i>	<i>H</i>
Gir lions	0	0	0.038
African lions	0.04–0.11	0.015–0.30	0.45



For both measures, Gir lions had far less genetic diversity than African lions. For DNA fingerprints, Gir lions are almost as similar as identical twins in humans.

The most probable explanation for the low genetic diversity in Asiatic lions is that the population has been small (bottlenecked) for an extensive period ([Chapter 11](#)). They experienced a severe reduction in population size (to fewer than 20 individuals) in the early 1900s.

Surprisingly, captive populations of 'Asiatic' lions were found to have genetic diversity for allozymes. However, this proved to be due to inadvertent crossing with African lions.

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## Measuring genetic diversity

Genetic diversity has been measured for many different traits, including proteins, nuclear DNA loci, mitochondrial DNA (mtDNA), chloroplast DNA (cpDNA), deleterious alleles and continuously varying (quantitative) characters, and for chromosomes. Most new data are being generated for variation at the DNA level, as methods have improved and costs decreased.

### Proteins

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Extensive information on genetic diversity has been obtained using electrophoretic separation of proteins

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Allelic variation at loci coding for soluble proteins is distinguished using electrophoresis, which separates proteins according to their net charge and molecular weights in an electrical potential gradient ([Box 3.2](#)). However, only about 30% of changes in DNA result in charge changes in proteins, so this technique underestimates the full extent of genetic diversity.

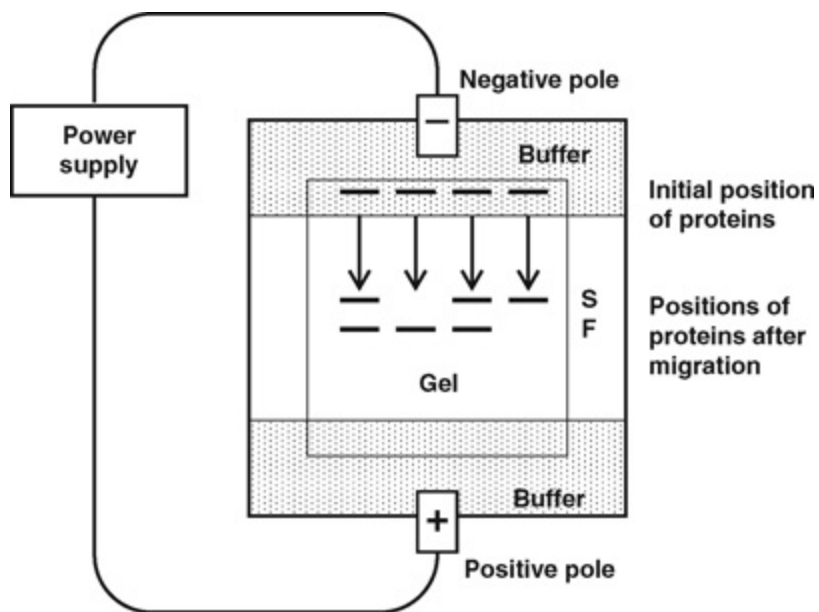
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**Box 3.2 Measuring genetic diversity in proteins using allozyme electrophoresis (see Leberg [1996](#); Schlötterer [2004](#))**

The sequence of amino acids making up a protein is determined by the sequence of bases in the DNA coding for that protein. Changes in the base sequence can replace amino acids in the protein. As five of the 20 naturally occurring amino acids are electrically charged (lysine, arginine and histidine [+], glutamic acid and aspartic acid [-]), about 30% of the DNA substitutions result in electrical charge changes in the resultant protein. These changes, as well as differences in molecular weights caused by base changes, can be detected by separating the proteins in an electrical potential gradient and subsequently visualized using a locus-specific histochemical stain. This process is termed **allozyme** electrophoresis. For example, if the DNA in alleles at a locus have the sequences:

	DNA	DNA with base substitution
Coding strand	...TAC GAA CTG CAA...	...TAC GAA <b>CCG</b> CAA...
mRNA	...AUG CUU GAC GUU...	...AUG CUU <b>GGC</b> GUU...
amino acid sequence	...met – leu – asp – val ...	...met – leu – <b>gly</b> – val ...

The protein on the right will migrate more slowly towards the anode in an electrical potential gradient, as a consequence of the substitution of uncharged glycine (gly) amino acid for negatively charged aspartic acid (asp).



A protein electrophoresis apparatus (after Hedrick [1983](#)). Soluble protein extracts are placed in spaced positions across the top of the separation gel. An electrical potential gradient applied to the gel causes the proteins to migrate through the gel. Proteins coded for by the same genetic locus but with different charges migrate to different positions (F–fast vs. S–slow), allowing identification of the different alleles at the locus. Proteins from specific loci are usually detected by their unique enzymatic activity, using a histochemical stain.



Protein electrophoresis is typically conducted using samples of blood, muscle, liver or kidney in animals, or leaves and root tips in plants, as these contain ample amounts and varieties of soluble proteins. Consequently, animals must be captured to obtain blood samples, or killed to obtain other tissue samples. These are unsuitable practices for endangered species. Soluble proteins are relatively fragile molecules and protein techniques, unlike DNA techniques, require fresh or fresh-frozen samples. This technique was used widely during the 1970s and 1980s, but has more recently been replaced by DNA-based methods, especially microsatellites (see [Box 3.3](#)).

## Collecting DNA samples for measuring genetic diversity

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Several methods are available for measuring genetic diversity in the DNA following non-invasive or 'remote' sampling and PCR amplification of DNA

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A major advantage of measuring DNA variation, as opposed to protein variation, is that samples can often be taken non-invasively, and genotypes identified following DNA amplification (see below). Any biological material containing DNA can be used to measure genetic diversity using modern molecular techniques (Avisé [2004](#); Schlötterer [2004](#)). For example, DNA can be obtained from shed hair, skin, feathers, faeces, urine, egg shell, fish scales, blood, tissues, saliva and semen in animals, and from tissue and pollen in plants. Museum skins, preserved tissues and plant herbarium material may provide adequate material. The only requirements are that the sample contains some undegraded DNA and that it is not contaminated with DNA from other individuals or closely related species.

Since extremely small samples of DNA (as little as the content of a single

cell) can be amplified millions of times by PCR (see below) only minute biological samples are now needed to conduct molecular genetic analyses.

## DNA amplification using PCR

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Very small quantities of DNA can be amplified using the polymerase chain reaction (PCR)

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Most current methods of measuring DNA diversity rely on the **polymerase chain reaction (PCR)** which allows laboratory amplification of specific DNA sequences from very small DNA samples ([Fig. 3.1](#)).

To amplify a DNA segment of interest, specific invariant (conserved) sequences on either side of the segment must be identified to design primers for the PCR reaction. The segment to be amplified is defined by, and lies between the two primers ([Fig. 3.1](#)). Copies of these sequences (oligonucleotides) are synthesized and used in the PCR reaction. Primer sequences can often be deduced from published sequence information for mitochondrial DNA (mtDNA), but must frequently be developed anew for nuclear loci, especially for microsatellites (see below).

## Measuring diversity at the DNA level

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Several methods are available for measuring diversity in DNA base sequences, with microsatellites being the method currently favoured

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**Box 3.3** describes many of the techniques available for directly or indirectly measuring DNA base sequence variation. DNA sequencing is routinely conducted, especially for taxonomic purposes. Microsatellites (variable number short tandem repeats: **Box 3.3**), have become the marker of choice for population studies. Microsatellites have advantages over other methods to measure DNA variation as they are highly variable, individual genotypes can be directly inferred and individuals can be identified (typed) following non-invasive sampling. They have the disadvantage that the primers must generally be developed anew for each locus for each species. Primers developed for one species may also work in a closely related species. For example, human primers usually work in chimpanzees, and some of the primers from domestic ruminants work in the endangered Arabian oryx.

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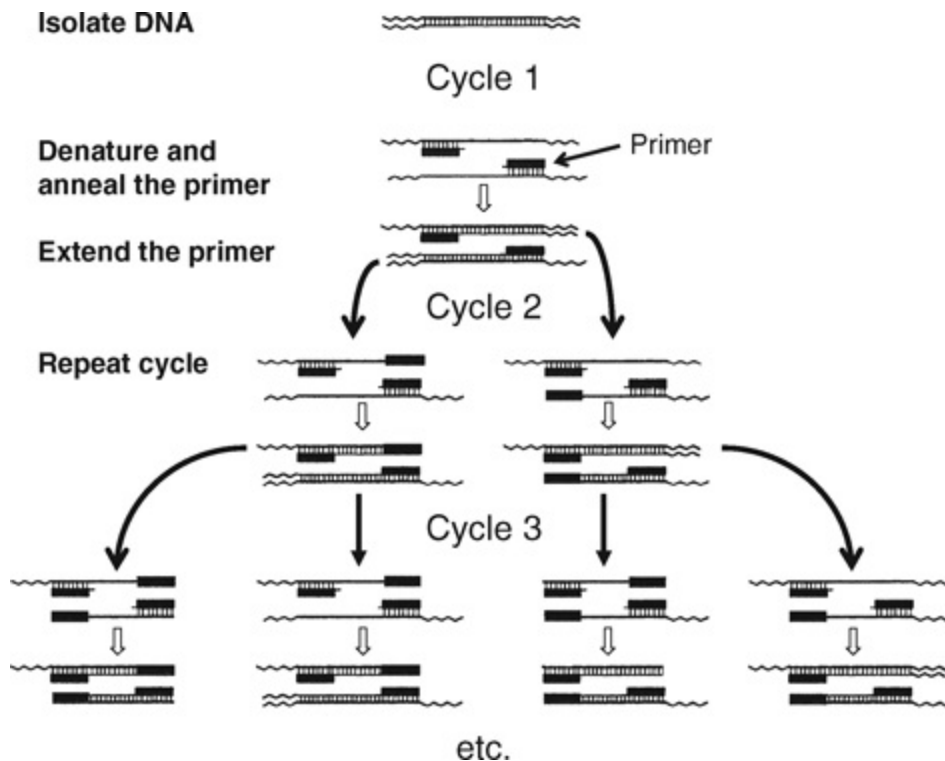
**Box 3.3 Techniques for measuring genetic diversity in DNA (Avisé 2004; Schlötterer 2004; Behura 2006)**

**Microsatellites (simple sequence repeats: SSR, or short tandem repeats: STR)**

Microsatellite loci are tandem repeats of short DNA sequences, typically 1–5 bases in length. For example, the DNA base sequence CA with 7 and 9 repeats (alleles A<sub>1</sub> and A<sub>2</sub>) are shown. CA repeats are found in many species. The number of microsatellite repeats is highly variable due to ‘slippage’ during DNA replication. The double-stranded DNA sequence of three genotypes, two different homozygotes and a heterozygote, are illustrated below along with their banding patterns following electrophoresis on a sequencing gel. **X** and **Y** are invariant (conserved) DNA sequences (primer sites) flanking the microsatellite repeat.

$A_1A_1$	$A_1A_2$	$A_2A_2$
<b>X</b> CACACACACACACAY	<b>X</b> CACACACACACACACAY	<b>X</b> CACACACACACACACAY
<b>X</b> GTGTGTGTGTGTGY	<b>X</b> GTGTGTGTGTGTGTGY	<b>X</b> GTGTGTGTGTGTGTGY
<b>X</b> CACACACACACACAY	<b>X</b> CACACACACACACAY	<b>X</b> CACACACACACACACAY
<b>X</b> GTGTGTGTGTGTGY	<b>X</b> GTGTGTGTGTGTGTGY	<b>X</b> GTGTGTGTGTGTGTGY
Fragment sizes on a gel (The samples loaded at top, migration is down the page, with smaller fragments coded for by the $A_1$ allele migrating furthest)		
_____	_____	_____
_____	_____	_____

Microsatellite diversity is detected by amplifying DNA using PCR (Fig. 3.1 above). The resulting DNA fragments are separated according to size using electrophoresis on acrylamide or agarose gels. After separation, the fragments are detected by either (1) use of fluorescently labelled primers and running the PCR products on a DNA sequencing machine (see chapter frontispiece), (2) use of radioactively labelled primers and autoradiography of gels (Chapter 4 frontispiece) or (3) staining gels with ethidium bromide (a DNA stain). If an individual is heterozygous for two microsatellite alleles with different numbers of repeats, then two different sized bands will be detected, as shown above.



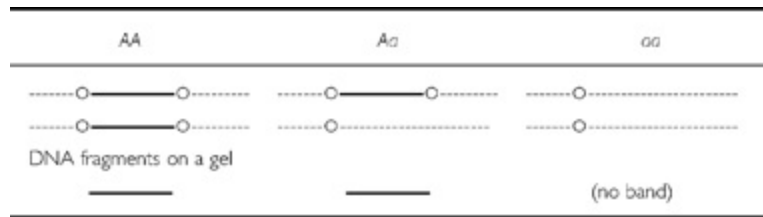
**Fig. 3.1** Non-invasive sampling of DNA and use of the polymerase chain reaction (PCR) to amplify DNA. PCR is used to amplify (generate

multiple copies of) DNA from tiny samples. PCR is essentially a test-tube version of natural DNA replication, except that it only replicates the DNA region of interest. DNA is extracted and purified from the biological sample and added to a reaction mix containing all the necessary reagents. These include DNA oligonucleotide primers, a heat-resistant DNA replicating enzyme (*Taq* polymerase), magnesium, the four DNA nucleotides and buffer. The primers are homologous to the conserved DNA sequences on either side of (flanking) the DNA sequence to be amplified (i.e. the locus of interest). The *Taq* polymerase enzyme replicates DNA, the nucleotides are the building blocks of the new DNA strands and magnesium and buffer are required for the enzyme to work. Repeated temperature cycles are used to denature the DNA (separate the strands), allow the DNA primers to attach to the flanking sequences (anneal) and to replicate the DNA sequence between the two primers (extend). Each cycle doubles the quantity of DNA of interest.

Microsatellites typically measure genetic variation for loci that are neutral (not exposed to selection), since the tandem repeats are usually located in non-coding segments of the DNA.

### **AFLP: amplified fragment length polymorphism**

DNA is cut with a restriction enzyme and short synthetic DNA fragments (adapters) of known sequence are attached to the cut ends. Since the enzyme cuts the DNA wherever there is an enzyme-specific nucleotide sequence, the DNA is cut into variable lengths and variability in this pattern among individuals reflects DNA sequence variation. PCR is carried out using primers that match the known adapter sequence plus additional 'selective' nucleotides. The method produces a multilocus DNA fingerprint apparent as either band presence (dominant) or absence (recessive). A single AFLP locus is illustrated below, along with the pattern observed for the three genotypes on a gel. 'o' represents a site in the DNA that has a sequence that is cut by a specific restriction enzyme and the solid line is the fragment of DNA amplified by PCR. A is dominant and *a* recessive (absence of primer sites). Many such loci are usually typed simultaneously.



## SNP: single nucleotide polymorphism

A position in the DNA of a species at which two or more alternative bases occur at appreciable frequency (>1%) is referred to as a single nucleotide polymorphism. These can be detected by a wide variety of techniques including sequencing, allele specific PCR or using DNA microarray chips (many short DNA sequences attached to a slide) (Black & Vontas 2007; Kim & Misra 2007). For example, 90% of the known SNP differences between two yeast strains were detectable on a single DNA chip (Gresham *et al.* 2006). SNPs are most practical for species that have been sequenced, or for species closely related to them. Their utility in conservation genetics is increasing as more species are sequenced (Chapter 10).

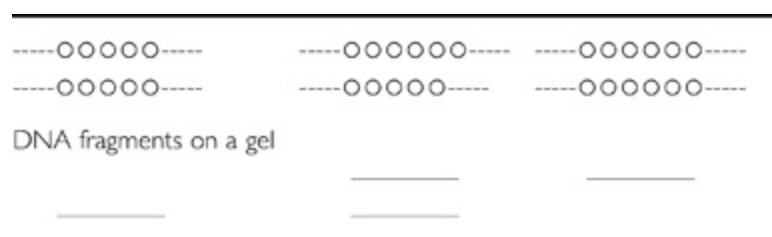
## RAPD: randomly amplified polymorphic DNA

For this technique, random primer sequences (rather than specific ones used in microsatellites), usually 10–20 base pairs in length, are used for PCR reactions on nuclear DNA samples. These yield a series of DNA fragments, which are separated on agarose or sequencing gels. Typically, several fragments in the 100–200 base size range amplify, so that several bands are detected for each primer (Fig. 21.8). If there is variation in the priming sites in the DNA, then some bands will reveal a presence–absence pattern similar to that for AFLPs above. Inheritance is dominant (presence) / recessive (absence). RAPDs assay many loci without the need to sequence the genome and design specific primers. Their disadvantages are the dominant mode of inheritance and concern about the repeatability of results. They have been largely replaced by AFLPs. Longer primer sequences generally provide higher repeatability. RAPDs have been widely used in plants, but less so in animals.

## DNA fingerprints (minisatellites, or variable number tandem repeats: VNTR)

Variable number tandem repeat sequences are found throughout the genome of humans and other eukaryotes. These minisatellite sequences have core repeat sequences with lengths in the range of 10–100 bases (i.e. they are larger than microsatellites). Typing of individuals for DNA fingerprints results in a ‘barcode’, where each individual is usually unique. To identify minisatellites, DNA is purified, cut with a restriction enzyme that cleaves outside the repeat, releasing the minisatellite DNA fragment, and the fragmented DNA separated according to size on an agarose gel. The two strands of the DNA fragments are separated (denatured) and transferred to a membrane (Southern blotting). The membrane with attached DNA is placed in a solution containing many copies of single-stranded radioactively labelled DNA of the specific core repeat sequence (probed). Radioactively labelled core sequences attach (hybridize) to minisatellite fragments on the membrane by complementary base pairing. Single-stranded unhybridized probe DNA is washed away, the membrane is dried and the position of minisatellites is revealed by autoradiography.

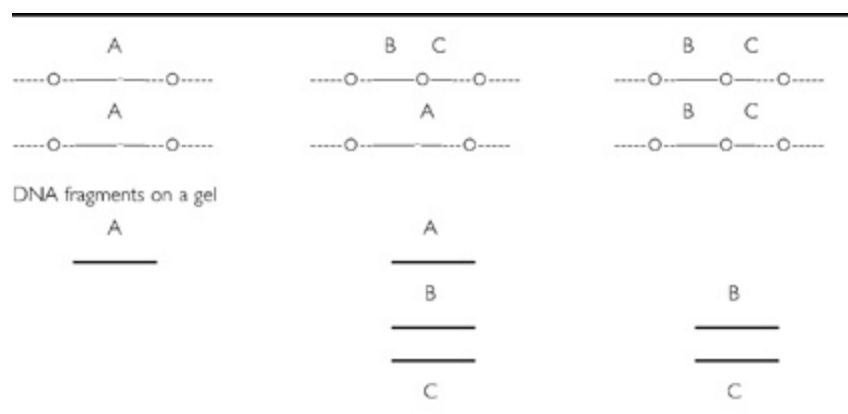
The number of repeats is highly variable, such that each individual in outbreeding species normally has a unique DNA fingerprint (apart from identical twins). Three genotypes for a single minisatellite locus are illustrated below, along with their banding patterns on a gel. ‘o’ represents a single repeat of the core sequence. Many such loci are typed simultaneously, resulting in a pattern of bands akin to a barcode ([Box 3.1](#)).



DNA fingerprints are highly variable, assess nuclear DNA variation over a wide range of loci and do not require a prior knowledge of DNA sequence in the species being typed. However, individual loci are not normally identifiable, as the fragments derive from many different places in the genome and typing requires considerable amounts of DNA. DNA fingerprints have now been replaced by methods that allow non-invasive sampling followed by PCR, such as microsatellites or AFLPs.

## RFLP: restriction fragment length polymorphism

For this method, DNA is purified, cut with a restriction enzyme and run on a gel to separate fragments of different size. The DNA strands are separated and transferred to a membrane, and the membrane dried. The membrane is placed in a solution containing many copies of single-stranded, radioactively labelled (typically  $^{32}\text{P}$ ) segments of DNA (probe) for the locus in question. After complementary base pairing, unhybridized single-stranded probe molecules are washed off, the membrane is dried and then autoradiographed. If there is variation in the DNA sequence at the restriction enzyme cutting site, then different sized fragments will be evident on the autoradiograph as shown below. 'o' represents sequences cut by the particular restriction enzyme used (a dash in the corresponding position indicates absence of the cut site) and the region recognized by the probe is shown as a solid line.



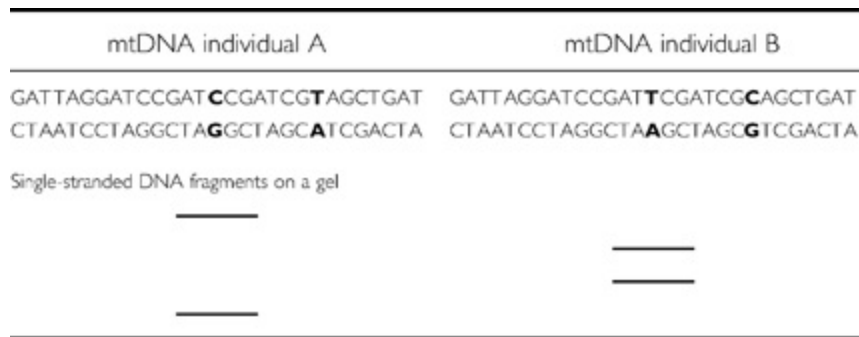
RFLP show co-dominant inheritance, they track variation in known genes and are moderately variable. As they require large amounts of DNA and cannot be typed following non-invasive sampling, they have been replaced by more convenient PCR-based methods.

## SSCP: single-strand conformational polymorphisms

Genetic diversity among PCR products from different individuals can be detected without sequencing by using SSCP. In this procedure, the two complementary strands of the DNA from the PCR product are separated at high temperatures (denatured), immediately cooled, and the single-stranded products subjected to electrophoresis in a polyacrylamide gel, at low temperature. Under these conditions, the single DNA strands fold



upon themselves in a sequence-specific manner. These molecules migrate in the gel according to both their size and their conformation. This method has been applied to detection of genetic diversity in mtDNA, and some nuclear loci (especially MHC loci). Examples of two mtDNA sequences (haplotypes) and their mobilities following SSCP are shown (after Smith & Wayne 1996). Note that there is a band for each of the single complementary DNA strands.



## DNA sequencing

The most direct means for measuring genetic diversity is to determine the sequences of bases, using DNA sequencing machines. Until recently, this was primarily used for taxonomic purposes, where mtDNA and/or nuclear loci were sequenced for a small number of individuals. However, technical improvements have markedly reduced the cost and time taken to sequence DNA, as is evident from the Human Genome Project and equivalent endeavours for many other species. Entire genomes have now been sequenced for many species and genome-wide analyses provide powerful new methods for understanding species' histories and biology (Chapter 10).

The characteristics of different molecular techniques for measuring genetic diversity are compared in Table 3.2. The mode of inheritance is an important determinant of the utility of a technique. Co-dominant inheritance is most desirable as this allows all genotypes to be distinguished. Methods that reveal higher levels of genetic diversity provide greater precision for most uses in conservation biology. For example, they provide more powerful comparisons

of threatened and non-threatened species, better discrimination in parentage, etc. Microsatellites reveal much higher levels of genetic diversity per locus than allozymes, while RAPDs, AFLPs and DNA fingerprints allow many more loci to be surveyed than is usually possible for allozymes. Many SNPs can be typed in species that have been sequenced.

**Table 3.2** *Characteristics of different molecular methods for assessing genetic diversity*

Method	Source	Non-invasive sampling	Cost	Development time <sup>a</sup>	Inheritance
Allozymes	blood, kidney, liver, leaves	No	Low	None	Co-dominant
Microsatellites	DNA	Yes	Moderate	Moderate	Co-dominant
AFLP	DNA	Yes	Moderate	Short	Dominant
SNP	DNA	Yes	Moderate–high	Considerable	Co-dominant
DNA fingerprints	DNA	No	Moderate	Short	Dominant
RAPD	DNA	Yes	Low–moderate	Short	Dominant
SSCP	DNA	Yes	Moderate	Moderate	Co-dominant
DNA sequencing	DNA	Yes	High	None	Co-dominant

<sup>a</sup> Indication of time taken to develop the technique so that genotyping can be done for threatened species.

## Mitochondrial DNA (mtDNA)

Mitochondrial DNA is maternally inherited in most species. It is used widely to assess taxonomic relationships and differences among populations within species

Mitochondria contain small circular haploid DNA molecules that are

maternally inherited (mother to offspring) in most species and usually transmitted without recombination (Barr *et al.* 2005). Mitochondrial DNA is relatively abundant, as there are many mitochondria per cell, and easy to purify. Genetic diversity in this DNA can be detected by sequencing, SSCP or cutting with restriction enzymes (RFLP) (Box 3.3). DNA primers that work for most species are now available for several loci in mtDNA. These loci can be amplified by PCR and the products sequenced. Sequencing of mtDNA has advantages over other techniques in that it can be done following non-invasive sampling, that mtDNA has a high mutation rate in animals and is highly variable, and that it can be used to specifically trace female lines of descent, or migration patterns. Its disadvantages are that it traces only a single maternally inherited unit. We will defer further consideration of mtDNA variation to Chapters 16 and 21, as its main conservation uses are in resolving taxonomic uncertainties, defining management units and in helping understand important aspects of species biology.

The equivalent organellar DNA in the chloroplasts of plants can be used for similar purposes.

## Deleterious alleles

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Recessive deleterious alleles are exposed by matings among relatives (inbreeding)

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As the majority of mutations are from a functional allele to a less functional state, part of the genetic diversity present in populations is due to deleterious alleles, such as those causing genetic diseases in humans and other species. Detection of this form of genetic diversity requires identification of genetically based deformities and malfunctions. However,

deleterious alleles are usually rare and predominantly recessive, and usually concealed in the heterozygous state. Consequently, deliberate inbreeding has been used to expose deleterious alleles in laboratory animals and domestic plants ([Chapter 13](#)). Special techniques are also available in fruit flies to make entire chromosomes homozygous (instant complete inbreeding) and to define the number of lethal, sub-lethal and deleterious alleles. (Lewontin 1974). Consequently, much of the precise data on deleterious alleles come from fruit fly species. Since inbreeding is deleterious, it cannot be used deliberately to estimate the frequency of deleterious alleles in endangered species. Thus, the limited available data come from inadvertent or unavoidable inbreeding of these species.

## Quantitative characters

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Variation for quantitative characters is due to both genetic and environmental causes. A genetic component is demonstrated by response to artificial selection, or by resemblances among relatives that are not due to a common environment

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Individuals vary in reproduction and survival (e.g. age at first reproduction, litter size, seed set, lifetime reproductive output, longevity). These traits and other measurable characters, such as height, weight, etc., are referred to as **quantitative characters**.

The existence of variation in quantitative characters is obvious in humans; we differ in height, weight and shape. However, this variation is due to both genetic and environmental causes. Artificial selection or statistical analyses of resemblances among relatives can be used to demonstrate the genetic component of differences among individuals for quantitative characters

(Chapter 5).

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Quantitative genetic variation for life history traits is the major determinant of evolutionary potential

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Evolutionary potential is most directly measured by quantitative genetic variation for reproductive fitness (Franklin 1980). Unfortunately, quantitative genetic variation is the most difficult to measure and the aspect of genetic diversity for which we have least information in threatened species (Chapter 5). Molecular measures of genetic diversity typically show little relation to quantitative genetic variation.

## Chromosomes

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The number, size and shape of chromosomes among individuals within species are usually the same, but chromosomes often differ between species

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The primary use of chromosomal diversity is to differentiate species (Benirschke & Kumamoto 1991). Species usually differ in the number, shape and/or banding patterns of their chromosomes. For example, the Chinese and Indian muntjac (barking deer) appear so similar that some authorities considered them to belong to the same species. However, the Chinese

muntjac has 46 chromosomes and the Indian muntjac six in males and seven in females (Ryder & Fleischer 1996).

Chromosomes are characterized by developing pictures of arranged chromosomes (karyotypes) from dividing cells. Animal somatic cells may be cultured from blood or skin biopsies, and germ line cells from testis biopsies. In plants, root tips provide somatic cells while flower buds provide germ line material. Differential staining methods can also be used to reveal chromosomal bands. This may reveal variation among individuals within species. For example, variants with different gene orders (inversions) are found in a wide diversity of species, including humans (White 1973; Levy *et al.* 2007). Genome sequencing has also revealed further chromosomal variation within species, including inversions, deletions and copy number variation (Hoffmann *et al.* 2004; Levy *et al.* 2007). Individuals heterozygous for some types of chromosome rearrangements have reduced fertility, so animals with reproductive difficulties in captive breeding programs may be examined cytologically. Populations of plants may differ in ploidy level (the number of sets of chromosomes:  $2n$  diploid,  $4n$  tetraploid, etc.). Further information on chromosomes is provided in Chapter 16.

## Extent of genetic diversity

Large populations of naturally outbreeding species usually possess considerable stores of genetic diversity, as detected by DNA, protein, deleterious allele, and quantitative character analysis

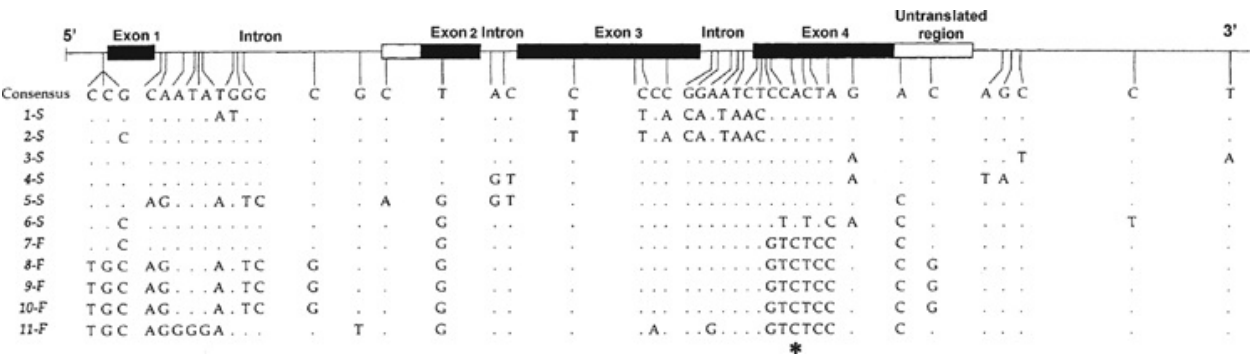
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Large populations of naturally outbreeding species usually possess considerable stores of genetic diversity, as detected by DNA, protein, deleterious allele, and quantitative character analysis

# Nuclear DNA

There is widespread genetic diversity in DNA sequences within outbreeding species

The first study of DNA sequence variation at a locus within a population was by Kreitman (1983) for the alcohol dehydrogenase (*Adh*) locus in fruit flies (Fig. 3.2). Among 11 samples, there were 43 variable sites across 2379 base pairs. The majority of base changes (42/43) do not result in amino acid substitutions (i.e. they are **silent substitutions**) as they were in non-coding regions of the locus (**introns**), or were in the third position of triplets coding for amino acids. Most **exons** (regions coding for amino acids) showed low variation. However, polymorphism was highest around the one polymorphic site causing an amino acid polymorphism in the *Adh* protein, a site where selection favours genetic diversity (Chapter 9). Sequence variation at the *Adh* locus in two outbreeding plant species from the *Brassica* family was, if anything, even higher than that found in the fruit fly (Liu *et al.* 1998).



**Fig. 3.2** DNA sequence variation found among 11 samples of the alcohol dehydrogenase locus from wild populations of fruit flies (after Li & Graur 1991 from data of Kreitman). Only polymorphic sites are illustrated.

Differences from the most common (consensus) sequence in the top row are given letters, while dots indicate identity with the consensus sequence. The asterisk in exon 4 indicates the site of the lysine for threonine replacement that is responsible for the polymorphism in allozyme electrophoretic mobility.

In humans, SNPs occur on average every 1000 bases, and over 9 million have been recorded (Kim & Misra [2007](#)). An average locus contains 126 single base polymorphisms, 46 of which are common (minor allele frequency of 5% or more) and five of which are found in the coding region (Crawford *et al.* [2005](#)). Given that there are about 20 000 coding loci in mammals and much sequence variation in DNA outside coding loci, there is an enormous amount of genetic diversity at the DNA level in most species. Few data are so far available on SNP variation for endangered species.



Fruit fly

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Most of the polymorphisms at the DNA level have little functional significance, as they occur in non-coding regions of the genome, or do not alter the amino acid sequence of a protein

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The highest levels of genetic diversity in DNA are typically found for base positions with little functional significance; ones that either do not code for functional products, or where substitutions do not change the function of the molecule. Conversely, the lowest genetic diversity is usually found for



functionally important regions of molecules, such as the active sites of enzymes (Hartl & Clark 2007). For example, in humans there are fewer SNPs in coding (4%) than non-coding regions (96%) (Crawford *et al.* 2005). Much of the DNA in an organism does not code for functional products (Chapter 10). Changes in the base composition in these regions often have little impact on fitness. They include regions between loci as well as regions within loci (introns) that are transcribed, but not translated. Further, about 70% of base substitutions within loci are silent so they are likely to be subjected, at most, to only very weak natural selection. Some regions of polypeptide chains are cleaved off before a functional protein is produced. These regions, and many regions outside the active sites of enzymes and proteins, are constrained only by limited functional demands.

There are two major exceptions to the generalization that polymorphism is lowest in regions with important functions. These are the major histocompatibility complex (a large family of genes that play an important role in the vertebrate immune system and in fighting disease), and self-incompatibility loci in plants. Both regions have very high levels of genetic diversity due to natural selection (Chapters 2 and 9).

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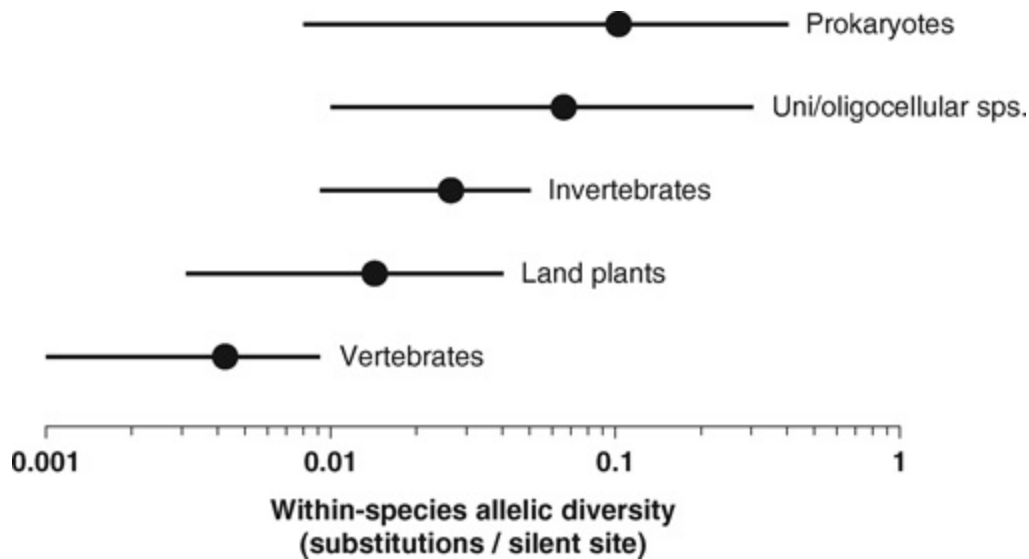
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DNA sequence variation within species is greatest in prokaryotes, followed by unicellular eukaryotes, invertebrates and land plants, with vertebrates lowest

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Levels of DNA sequence variation within species differ among major taxa, with levels increasing with population sizes for species in the taxa (Fig. 3.3).



**Fig. 3.3** Average levels of within-species DNA nucleotide variation for silent sites across major taxa (Lynch 2006). Means and ranges are shown. Note that the scale is logarithmic.

## Extent of microsatellite variation

Microsatellites show high levels of genetic diversity. They provide one of the most powerful and practical means for surveying genetic diversity in threatened species

Microsatellites have been used to measure genetic diversity in a wide variety of species, many of them endangered. They typically show very high levels of polymorphism and many alleles per locus. For example, CA repeats (Box 3.3) are common and often vary in repeat number within populations; alleles with 10 repeats vs. 12 vs. 15, etc. may segregate in the same population. Such diversity has been found in all species so far examined. For example, data from a survey of microsatellite variation at eight loci in 39 wild chimpanzees detected an average of 5.75 alleles per locus (A) and an average

heterozygosity of 0.70 (Table 3.3). High levels of genetic diversity in large populations of a variety of non-endangered species are evident from the examples in Table 3.5 below.

**Table 3.3** *Microsatellite variation at eight loci in 39 wild chimpanzees from Gombe National Park, Tanzania. Allele frequencies are given, along with the proportions of individuals heterozygous (H) for each locus and the average number of alleles per locus (A)*

	Locus								
Allele	D19S431	D9S905	D18S536	D4S243	D15S48	D9S922	D2S1326	D9S302	
1	0.458	0.086	0.412	0.197	0.219	0.016	0.258	0.071	
2	0.097	0.186	0.074	0.224	0.516	0.210	0.182	0.014	
3	0.042	0.057	0.074	0.210	0.266	0.064	0.061	0.057	
4	0.014	0.243	0.265	0.013		0.290	0.288	0.014	
5	0.028	0.429	0.176	0.132		0.355	0.182	0.071	
6	0.361			0.224		0.064	0.030	0.100	
7								0.586	
8								0.029	
9								0.057	
H	0.647	0.712	0.718	0.799	0.615	0.737	0.780	0.629	Av. H = 0.705
									A = 5.75

Source: After Constable *et al.* (2001).

**Table 3.5** *Levels of microsatellite genetic diversity in a range of threatened and taxonomically related, non-threatened taxa. Average number of alleles per locus (A) and heterozygosity (H) are given for polymorphic loci. Globally threatened, or previously threatened, species (or sub-species) are placed adjacent to the most closely related, but non-threatened species (or sub-species) for which data are available*

Threatened species	A	H	Non-threatened species	A	H	Reference
African wild dog	3.5	0.56	Domestic dog	6.4	0.73	1
Black rhinoceros	4.2	0.69	African buffalo	8.6	0.73	2,3
Cheetah	3.4	0.39	Puma	4.9	0.61	1
Chimpanzee		0.64	Human		0.78	1
Ethiopian wolf	2.4	0.21	Coyote	5.9	0.68	1
Giant panda	3.7	0.44	Brown bear	6.8	0.66	4,5
Mexican wolf	2.7	0.42	Gray wolf	4.5	0.62	1
Long-footed potoroo	3.7	0.56	Allied rock wallaby	12.0	0.86	1
Bridled nail-tail wallaby	11.6	0.83				1
Northern hairy-nosed wombat	2.1	0.32	Southern hairy-nosed wombat	5.9	0.71	1
Mariana crow	1.8	0.16	American crow	6.0	0.68	1
Mauritius kestrel	1.4	0.10	European kestrel	5.5	0.68	1
Seychelles kestrel	1.3	0.12	Greater kestrel	4.5	0.59	1
Komodo dragon	4.0	0.31	American alligator	8.3	0.67	1
Honduras mahogany	9.7	0.55	Royal mahogany	9.3	0.67	1

*References:* 1, Frankham (2000b); 2, O'Ryan *et al.* (1998); 3, Brown & Houlden (1999); 4, Lu *et al.* (2001); 5, Waits *et al.* (2000).



Chimpanzee

Comparisons of genetic diversity among species for microsatellites are not as straight forward as for allozymes. Microsatellite mutation rates differ among species and microsatellites with more repeats have more variation than smaller ones. Further, primers developed for one species can lead to lower levels of variation being detected when used for other species (Primmer *et al.* 1996).

## Extent of protein variation

There is extensive genetic diversity at protein-coding loci in most large populations of outbred species. On average 28% of loci are polymorphic and 7% of loci are heterozygous in an average individual, as assessed by electrophoresis

Most non-threatened species with large population sizes show high levels of genetic diversity for allozymes. For example, in humans 32% of loci are polymorphic and an average individual is heterozygous for 6% of loci (Harris *et al.* 1977) and African lions show similar variability (Example 3.1). [Table 3.4](#) summarizes allozyme heterozygosities for several major taxa. Average heterozygosity within species ( $H$ ) is lower in vertebrates (6.4%) than in invertebrates (11.2%) or plants (23%), probably due to lower population sizes in vertebrates ([Chapters 8](#) and [11](#)).

**Table 3.4** *Allozyme genetic diversity in different taxa.  $H$  is the average heterozygosity within populations and  $G_{ST}$  is the proportion of the variation in allele frequencies that is attributable to variation among populations*

	$H$	$G_{ST}$
<b>Vertebrates</b>		
Mean	0.064	0.202
Mammals	0.054	0.242
Birds	0.054	0.076
Reptiles	0.090	0.258
Amphibians	0.094	0.315
Fish	0.054	0.135
<b>Invertebrates</b>		
Mean	0.113	0.171
Insects	0.122	0.097
Crustaceans	0.063	0.169
Mollusks	0.121	0.263
<b>Plants</b>		
Mean	0.113	0.224
Gymnosperms	0.160	0.068
Angiosperms	0.105	0.263

Sources: After Hamrick & Godt (1989); Ward *et al.* (1992).

Protein electrophoresis was the primary molecular tool for measuring genetic diversity from the late 1960s until the 1980s, with estimates of genetic diversity available for well over 1000 species (see Ward *et al.* 1992). It has now largely been replaced by microsatellite analyses, as they typically show much higher levels of genetic variation than allozymes (compare Example 3.1 and Table 3.5); microsatellites usually have five to ten alleles per polymorphic locus for large outbreeding populations, compared to about two for allozymes.

## Extent of deleterious allele variation

All outbred populations contain a 'load' of rare deleterious alleles that can be exposed by inbreeding

The extent of diversity in populations attributable to deleterious alleles is important in conservation because these alleles reduce reproductive fitness when they become homozygous following inbreeding ([Chapter 13](#)). Deleterious alleles are constantly generated by mutation and removed by selection. Consequently, all outbred populations contain deleterious rare alleles (**mutation load**: [Chapter 7](#)). Typically, these occur at frequencies of less than 1%. Rare human genetic syndromes, such as phenylketonuria, albinism and Huntington's disease are examples. Equivalent syndromes are found in wild populations of plants and animals. For example, mutations leading to a lack of chlorophyll are found in many plant species and a range of genetically based defects have been described in endangered animals (dwarfism in California condors, vitamin E malabsorption in Przewalski's horse, undescended testes and fatal heart defects in Florida panthers and hairlessness in red ruffed lemurs: Ryder [1988](#); Roelke *et al.* [1993](#); Ralls *et al.* [2000](#)).

Most deleterious alleles probably make no useful contribution to evolutionary potential. However, some are advantageous in different environments and a few show heterozygote advantage. In humans, heterozygotes for sickle-cell anaemia possess higher resistance to malaria than the normal homozygote, while most sickle-cell homozygotes die (semi-lethal) due to defective haemoglobin ([Chapter 9](#)).

## Extent of quantitative variation

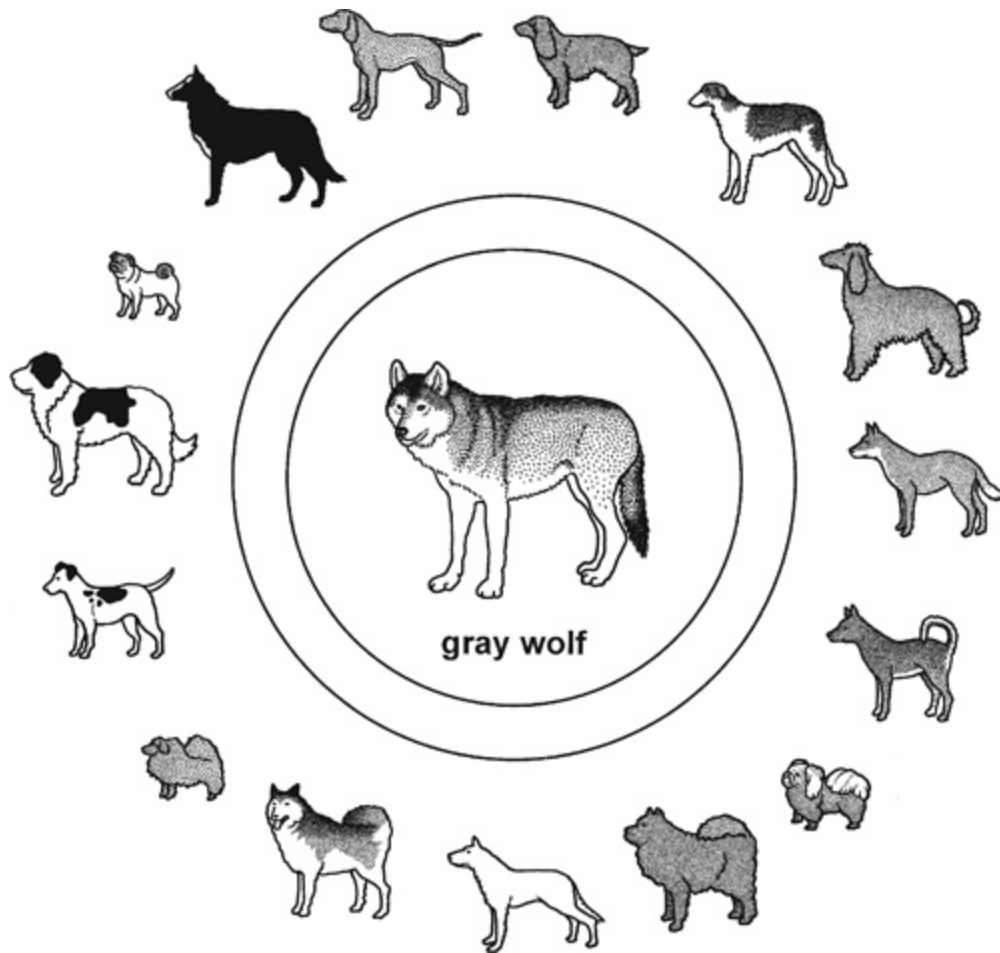
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Quantitative characters show extensive genetic diversity for essentially all characters in large outbreeding populations

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Quantitative genetic variation has been found for reproductive characters

(egg production in chickens, number of offspring in sheep, mice, pigs and fruit flies, and seed yield in plants, etc.), for growth rate (in cattle, pigs, mice, chickens, fruit flies and plants), for chemical composition (fat in animals, protein and oil in maize), for behaviour (in insects and mammals) and for disease resistance in plants and animals (Lewontin 1974). A familiar example is the diversity of breeds in domestic dogs which all belong to the same species (Fig. 3.4). With the exception of a few mutations, the variety of dog breeds reflects partitioning of extensive genetic diversity that was present in the ancestral wolves. Plant species also exhibit extensive genetic diversity. For example, the cabbage species has diversified to give cabbages (edible leaves), kohlrabi (edible roots), brussels sprouts (edible buds), broccoli (edible flowers), etc. (Diamond 1997). Species with large population sizes generally have vast evolutionary potential for quantitative characters (Chapters 5 and 6).



**Fig. 3.4** Diversity of dog breeds. All derive from the gray wolf.



Recently, exceptions to the universality of genetic diversity for quantitative characters have been discovered. Several populations of two rainforest fruit fly species in Australia lack quantitative genetic variation for desiccation resistance, but contain normal levels of quantitative genetic variation for other characters (Hoffmann *et al.* 2003; Kellermann *et al.* 2006). Conversely, several other fruit fly species show high levels of quantitative genetic variation for desiccation resistance.

## Low genetic diversity in threatened species and bottlenecked populations

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Threatened species usually have lower levels of genetic diversity than non-threatened species

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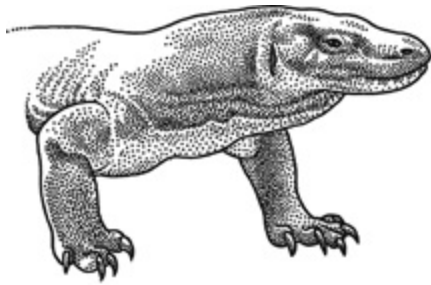
Of 170 threatened mammals, birds, fish, insects and plants, 77% had lower molecular genetic diversity (mainly for allozymes) than taxonomically related, non-threatened species (Spielman *et al.* 2004a). All 15 threatened taxa listed in Table 3.5 have lower heterozygosity than taxonomically related, non-endangered species for microsatellites. Threatened species of major taxa of plants and animals on average have about 65% the genetic diversity of non-threatened species. A similar conclusion also applies to comparisons involving DNA fingerprints, RAPD and AFLP.

---

Smaller or bottlenecked populations often have reduced genetic diversity

---

The abundant genetic diversity found in large populations contrasts with that found in many small or bottlenecked populations (Garner *et al.* [2005](#)). For example, the northern elephant seal that was hunted almost to extinction, but subsequently recovered from a population size bottleneck of 20–30, has no allozyme variation (Bonnell & Selander [1974](#)).



Komodo dragon



Cheetah

## Variation over space and time

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Spatial variation in genetic composition depends critically on rates of

gene flow, population sizes and selection

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As an approximation, the magnitude of genetic differentiation among animal populations is inversely correlated with dispersal ability ([Chapter 14](#)). This is evident from the  $G_{ST}$  values in [Table 3.4](#).  $G_{ST}$  measures the proportion of the total variation that occurs among, as opposed to within populations. Birds with high dispersal ability show the lowest  $G_{ST}$  values of all vertebrate groups. Similarly, insects which disperse easily show the lowest  $G_{ST}$  amongst invertebrates.

Plants typically display locally adapted races for quantitative genetic characters, and often show allozyme differences among populations, especially for self-fertilizing species. Clines, continuous changes in frequencies of genetic variants along environmental gradients (temperature, latitude or altitude), are common for morphological variation, fitness characters and chromosomal inversions (Hoffmann *et al.* [2004](#)). These often reflect gradients in natural selection affecting the genetic variants.

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Large populations typically show negligible short-term change in genetic diversity. Conversely, small populations typically lose genetic diversity rapidly over time

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Most allele frequencies in large populations are relatively stable for molecular genetic markers over the course of several decades. For example,

there were no consistent changes in allozyme frequencies in a wild fruit fly population over 25 years (over 250 generations) (Frankham & Loebel [1992](#)). In the same population, microsatellite allele frequencies were similar over four years (England *et al.* [2003](#)).

In contrast, small populations of threatened species typically lose genetic diversity over time. Analyses comparing old museum specimens with samples from current populations of species having suffered severe population bottlenecks show loss of genetic diversity in whooping cranes, Arabian oryx, Mauritius kestrels and nene (Glenn *et al.* [1999](#); Marshall *et al.* [1999](#); Groombridge *et al.* [2000](#); Paxinos *et al.* [2001](#); [Chapters 8](#) and [11](#)).



Whooping crane

A range of adaptive genetic variants, including inversions and allozyme variation at some loci, have altered with global climate change in recent years (Hoffmann *et al.* [2004](#); Umina *et al.* [2005](#)). Over much longer time spans, ancient brown bear DNA samples from Beringia showed that genetically and geographically distinct groups have replaced each other relatively often during the last 60 000 years (Barnes *et al.* [2002](#); Nicholls [2005](#)).

## Genetic differences among species

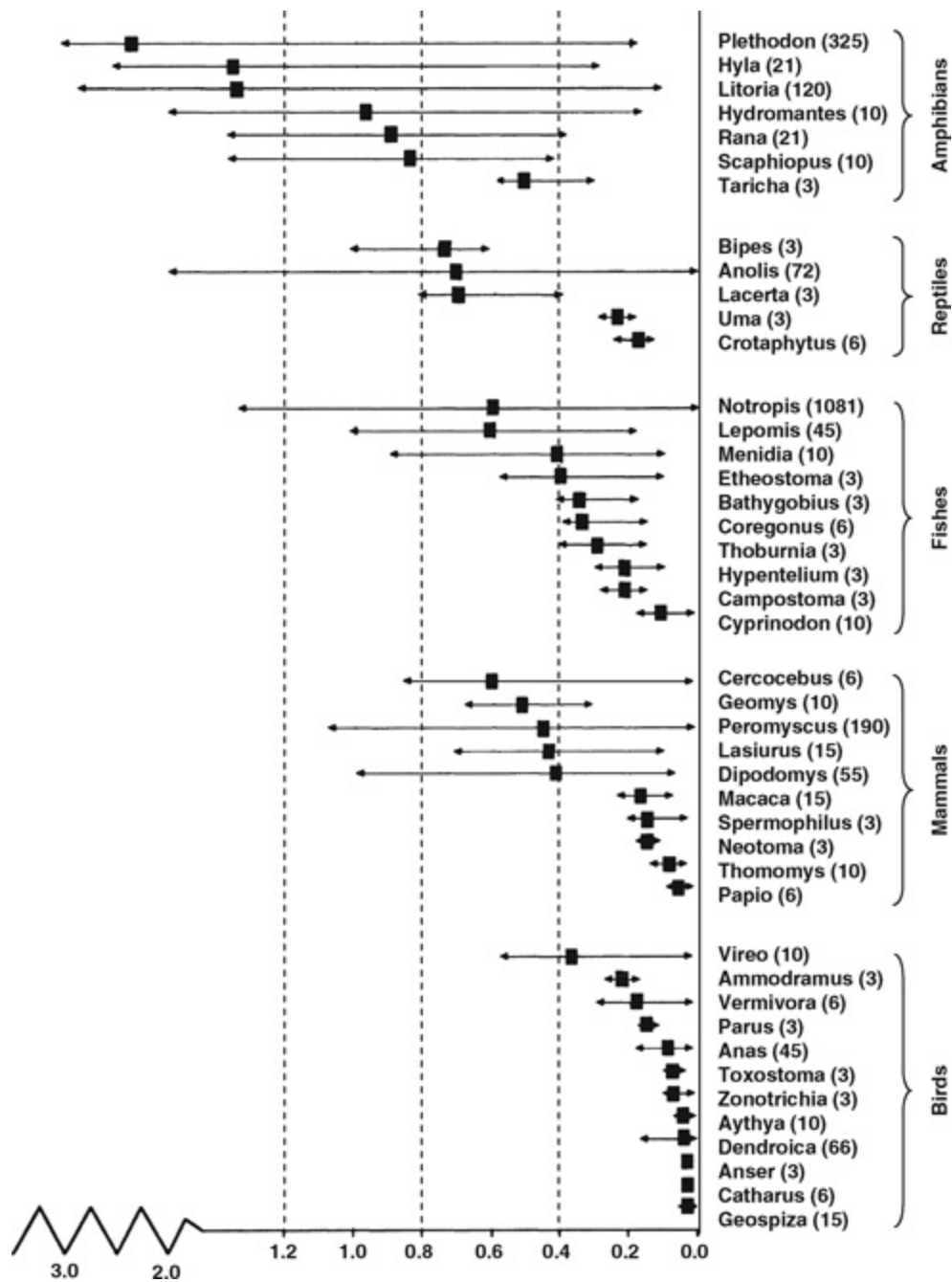
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The genetic differences among species roughly reflect their taxonomic divergence

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Trees of relationship among higher taxonomic ranks, derived from DNA or proteins, are often similar to those based upon morphology. However, the genetic divergence among species within different genera varies widely ([Fig. 3.5](#)), reflecting the somewhat arbitrary nature of taxonomic classifications. Genetic differentiation among bird species is on average less than that among species of other vertebrate groups (Johns & Avise [1998](#)). Species of fruit flies in the genus *Drosophila* typically show much greater genetic differentiation among species than do primates currently placed in different taxonomic families (Avise & Johns [1999](#)). Use of genetic diversity among taxa to resolve taxonomic uncertainties is considered in [Chapter 16](#).



**Fig. 3.5** Genetic differences among species in selected vertebrate genera (after Avise 2004 p.11). Means and ranges of genetic distances among species, based upon multilocus protein electrophoresis. This is an indirect measure of the amount of DNA sequence divergence among species. *Genetic distances among species within genera vary considerably among vertebrate classes.*

## Summary

1. Genetic diversity represents the essential raw material for species to evolve and adapt in response to changing environments.
2. Genetic diversity can be measured for DNA, proteins, deleterious alleles and quantitative characters.
3. Microsatellites are currently the most practical and informative of the molecular techniques for measuring DNA variation within populations.
4. Large populations of non-endangered species typically have extensive genetic diversity.
5. Most threatened species have reduced genetic diversity when compared to taxonomically related non-endangered species.

## Further reading

Avise (2004) *Molecular Markers, Natural History and Evolution*. Clear descriptions of the molecular methods for measuring genetic diversity and results achieved with them.

Hamrick & Godt (1989) Compilation of data on electrophoretic variation in plants and factors affecting it.

Hartl & Clark (2007) *Principles of Population Genetics*. Provides an easy-to-follow coverage of genetic diversity and its importance.

Hedrick (2005a) *Genetics of Populations*. Textbook with an extensive coverage of genetic diversity.

O'Brien (2003) *Tears of the Cheetah*. A popular book describing studies on molecular genetics of cheetahs and other species.

Schlötterer (2004) Excellent review of molecular markers, their history, uses, advantages and disadvantages.

Spielman *et al.* (2004a) Contains a comprehensive comparison of molecular genetic diversity in threatened and taxonomically related non-threatened species.

Ward *et al.* (1992) Compilation of data and analyses on electrophoretic

variation over more than 1000 animal species.

## Software

GEMINI: Software for testing the effects of genotyping errors and multitube approach for individual identification from microsatellites with poor-quality DNA (e.g. faeces) (Valière *et al.* 2002). <http://pbil.univ-lyon1.fr/software/Gemini/gemini.htm>

## Problems

- 3.1 Genetic diversity. Why is genetic diversity of importance in conservation biology?
- 3.2 Measurement of genetic diversity. What is the basis for using electrophoretic separation of proteins to measure the extent of genetic diversity?
- 3.3 Measuring genetic diversity. What are microsatellites?
- 3.4 Measuring genetic diversity. What is an AFLP?
- 3.5 Measuring genetic diversity. What is a RAPD?
- 3.6 Measuring genetic diversity. What is a DNA fingerprint?
- 3.7 Measuring genetic diversity. What genetic markers can be typed following non-invasive sampling?
- 3.8 Genetic diversity. What form of genetic diversity is most important for retaining evolutionary potential?
- 3.9 Levels of genetic diversity. How do major taxonomic groups compare in genetic diversity?
- 3.10 Levels of genetic diversity. How do endangered and taxonomically related non-endangered species compare in genetic diversity?

## Practical exercise: Measuring genetic diversity using microsatellites

This practical involves non-invasive sampling, PCR amplification and



microsatellite typing. Choose a species where microsatellites have been developed (e.g. see *Molecular Ecology Notes*). Microsatellite primers with fluorochromes for the loci to be used need to have been synthesized previously. Beware of cross-contamination during all stages of the procedure. This practical can conveniently be spread over two weeks, the first involving preparing hair, DNA extraction and DNA amplification. Microsatellite typing is done on a sequencing machine during the intervening period. In the second week, genotypes are illustrated on a computer screen and results analysed.

DNA can be obtained from hair from an interesting species (we used tammar wallabies). It can be collected from live animals, or frozen samples. Hairs with good roots are chosen and cut at a point about 3 mm above the bulb with a razor blade.

DNA can be extracted from the hair root by a simple boiling method, such as that of Sloane *et al.* (2000). Two loci labelled with different fluorochromes provide suitable material, though a single locus will suffice. Add microsatellite DNA primers, *Taq* polymerase, nucleotides and buffer, and amplify the DNA.

Separate amplified microsatellite fragments on a DNA sequencing machine and store images in an electronic form. View result and analyse data to obtain allele frequencies and heterozygosities.

## Chapter 4 Characterizing genetic diversity: single loci

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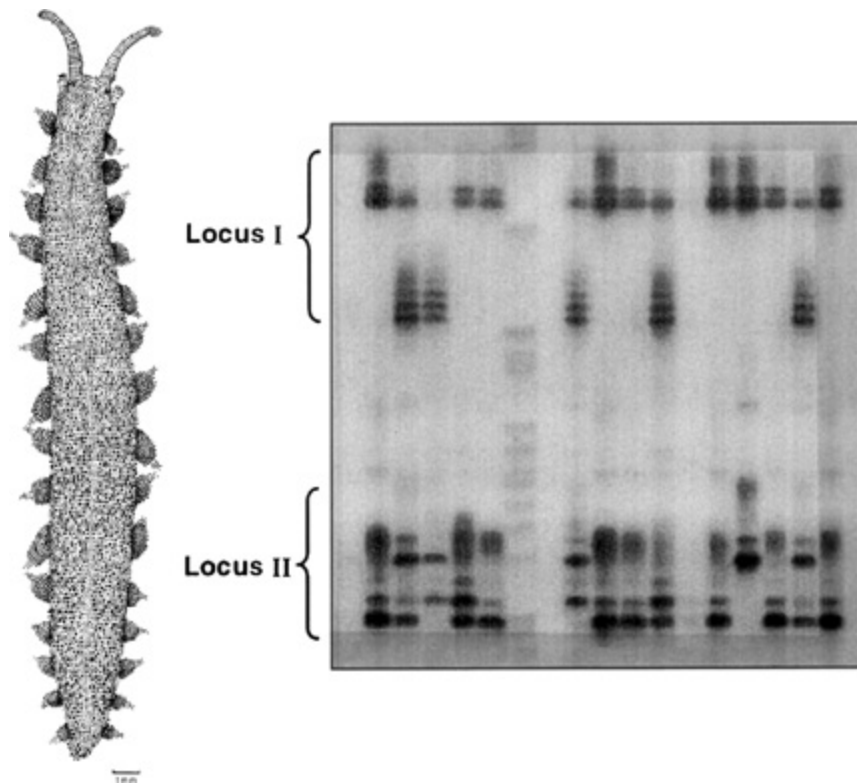
Heterozygosities and frequencies of alleles at individual loci are used to characterize genetic diversity in populations. Allele and genotype frequencies are in equilibrium under random mating when there are no other perturbing forces

### Terms

Allelic richness, autotetraploid, effective number of alleles, equilibrium, expected heterozygosity, gene diversity, haplotype diversity, Hardy–Weinberg equilibrium, hermaphrodite, linkage disequilibrium, nucleotide diversity, observed heterozygosity, polyploid, random mating, selfing, sex-linked, tetraploid

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An Australian velvet worm and an autoradiograph illustrating genetic diversity at microsatellite loci in this species

## Describing genetic diversity

Measures of genetic diversity are required to document losses of genetic variation, evolutionary changes and genetic differentiation of populations

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In the previous chapter, we described molecular techniques for determining the genotype of each individual from a sample of organisms within a population or species. We now describe the parameters that allow us to extrapolate from these samples to the entire population. These values are essential for comparisons among populations and species, and for predicting changes in the genetic composition of populations. Parameters for single loci are described in this chapter, while those for more complex, multilocus, quantitative characters are addressed in [Chapter 5](#).

## Frequencies of alleles and genotypes

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The genetic composition of a population is typically described in terms of heterozygosities, allele frequencies and number of alleles

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The information we collect provides details of the numbers of each genotype at a locus. This is illustrated for an egg-white protein locus in Scottish eider ducks, a species that was severely reduced due to harvest ([Table 4.1](#)). Genotype frequencies are simply calculated from the proportion of that type in the total sample (e.g. genotype frequency of FF =  $37/67 = 0.552$ ).



Eider ducks

**Table 4.1** *Numbers and frequencies of each genotype at an egg-white protein locus in eider ducks from Scotland, as determined by protein electrophoresis; F refers to the faster migrating allele and S to the slower*

	Genotypes			Total
	FF	FS	SS	
Numbers	37	24	6	67
Genotype frequencies	0.552	0.358	0.090	1.00

Source: Milne & Robertson (1965).

The information is usually reported in the form of allele frequencies, rather than genotype frequencies. We use the letters  $p$  and  $q$  to represent the allele frequencies for two alleles at the locus. The frequency of the F allele ( $p$ ) in the eider duck population is simply the proportion of all alleles examined which are F. As the ducks are diploid, we double the numbers of each homozygote, and the total number of individuals.

$$p = \frac{(2 \times \text{FF}) + \text{FS}}{2 \times \text{Total}} \quad (4.1)$$

The calculation in Example 4.1 shows that 73% of the alleles at this locus are F and 27% are S.

Similar procedures are applied to obtain allele frequencies when there are

more than two alleles at a locus, as found for many microsatellite loci. Numbers of each genotype at a microsatellite locus with three alleles in the endangered Hawaiian Laysan finch are given in

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**Example 4.1 Calculation of F and S allele frequencies at an egg-white protein locus in eider ducks**

The frequency for the F allele  $p$  is obtained as follows:

$$p = \frac{[(2 \times 37) + (1 \times 24)]}{(2 \times 67)} = 0.73$$

and that for S ( $q$ ) as:

$$q = \frac{[(2 \times 6) + (1 \times 24)]}{(2 \times 67)} = 0.27$$

The sum of the relative frequencies of all alleles must equal 1.00, i.e.

$$p + q = 0.73 + 0.27 = 1.00$$

Allele frequencies may also be reported as percentages.

---

[Table 4.2](#), and the method for estimating the frequency of one of the alleles is shown in Example 4.2.

**Table 4.2** *Numbers of each genotype at a microsatellite locus with three alleles in the endangered Hawaiian Laysan finch. The allelic designations 91, 95 and 97 are the sizes, in base pairs, of the amplified PCR fragments*

	Genotypes						Total
	91/91	91/95	91/97	95/95	95/97	97/97	
Numbers	7	10	8	5	11	3	44

Source: Tarr *et al.* (1998).

### Example 4.2 Estimating allele frequencies at a locus with three alleles in the endangered Laysan finch

The frequency of the 91 allele ( $p$ ) is obtained by counting the number of 91 alleles (twice the number of the 91/91 genotype, plus the numbers of the 91/95 and 91/97 genotypes) and dividing by twice the total number of individuals, as follows:

$$p = \frac{[(2 \times 7) + 10 + 8]}{(2 \times 44)} = 0.364$$

The frequencies of the 95 and 97 alleles, calculated in a similar manner, are 0.352 and 0.284, respectively. The frequencies of the three alleles sum to 1.000.

Heterozygosity is the measure most commonly used to characterize genetic diversity for single loci

**Observed heterozygosity ( $H_o$ )** is simply the number of heterozygotes at a

locus divided by the total number of individuals sampled. For example, the observed frequency of heterozygotes at the egg-white protein locus in eider ducks is  $24/67 = 0.36$  (Table 4.1). When there are more than two alleles at a locus the total number of heterozygotes is summed. For the Laysan finch microsatellites (Table 4.2), the observed heterozygosity is  $(10 + 8 + 11)/44 = 0.659$ . Average heterozygosity is typically used to compare the extent of genetic diversity among populations or species (Table 3.1). Generally, expected heterozygosity ( $H_e$ ), described later, is reported for outbreeding species, as it is less sensitive to sample size than observed heterozygosity.

We now deal with factors that influence the frequencies of alleles and genotypes, followed by consideration of the consequences of different mating systems.

## Hardy–Weinberg equilibrium

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In a large random mating population, allele and genotype frequencies at an autosomal locus attain equilibrium after one generation, when there are no perturbing forces (no mutation, migration or selection)

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Let us begin with the simplest case – that of a large population where mating is random and there is no mutation, migration or selection. In this case, allele and genotype frequencies attain equilibrium after just one generation and remain constant thereafter. This is referred to as the **Hardy–Weinberg equilibrium**, after its discoverers. The Hardy–Weinberg equilibrium is simple, yet crucial to conservation and evolutionary genetics. It provides a basis for detecting deviations from random mating, testing for selection, modelling the effects of inbreeding and selection and estimating the



allele frequencies at loci showing dominance.

We determine the relationship between allele and genotype frequencies using a simple mathematical model. Assume that we are dealing with a locus with two alleles  $A_1$  and  $A_2$  at relative frequencies of  $p$  and  $q$  ( $p + q = 1$ ) in a large random mating population. Imagine **hermaphroditic** marine organisms (both sperm and eggs released by each individual) shedding their gametes into the water, where sperm and eggs unite by chance ([Table 4.3](#)). Since the allele frequency of  $A_1$  in the population is  $p$ , the frequency of sperm or eggs carrying that allele is also  $p$ . The probability of a sperm carrying  $A_1$  uniting with an egg bearing the same allele, to produce an  $A_1A_1$  zygote, is therefore  $p \times p = p^2$  and the probability of an  $A_2$  sperm fertilizing an  $A_2$  egg, to produce an  $A_2A_2$  zygote is, likewise,  $q \times q = q^2$ . Heterozygous zygotes can be produced in two ways,  $A_1$  ovum  $\times$   $A_2$  sperm or  $A_2$  ovum  $\times$   $A_1$  sperm, each with a frequency of  $pq$ , so their overall expected frequency is  $2pq$ . Consequently, the expected genotype frequencies for  $A_1A_1$ ,  $A_1A_2$  and  $A_2A_2$  zygotes are  $p^2$ ,  $2pq$  and  $q^2$ , respectively. These are the **Hardy–Weinberg equilibrium** genotype frequencies.

**Table 4.3** *Genotype frequencies resulting from random union of gametes at an autosomal locus*

			Ova	
			A <sub>1</sub>	A <sub>2</sub>
			p	q
Sperm	A <sub>1</sub>	p	<b>p<sup>2</sup></b> A <sub>1</sub> A <sub>1</sub>	<b>pq</b> A <sub>1</sub> A <sub>2</sub>
	A <sub>2</sub>	q	<b>pq</b> A <sub>2</sub> A <sub>1</sub>	<b>q<sup>2</sup></b> A <sub>2</sub> A <sub>2</sub>

The resulting genotype frequencies in progeny are

A <sub>1</sub> A <sub>1</sub>	A <sub>1</sub> A <sub>2</sub>	A <sub>2</sub> A <sub>2</sub>
p <sup>2</sup>	2pq	q <sup>2</sup>

These are the **Hardy–Weinberg equilibrium** genotype frequencies.

If the frequencies of the alleles A<sub>1</sub> and A<sub>2</sub> are 0.9 and 0.1, then the Hardy–Weinberg equilibrium genotype frequencies are:

A <sub>1</sub> A <sub>1</sub>	A <sub>1</sub> A <sub>2</sub>	A <sub>2</sub> A <sub>2</sub>	Total
0.9 <sup>2</sup>	2 × 0.9 × 0.1	0.1 <sup>2</sup>	1.0
0.81	0.18	0.01	1.0

In a large population, there will be no net loss or gain of either allele and their frequencies are said to be in **equilibrium**. We can verify this by calculating the new allele frequencies ( $p_1$  and  $q_1$ ) for the progeny. Following Example 4.1, (but using simple algebraic terms) we calculate allele frequencies by counting the number of a particular allele and dividing by the total number of alleles. The frequency of A<sub>1</sub> in the progeny  $p_1$  is:

$$p_1 = \frac{(2 \times p^2) + 2pq}{2(p^2 + 2pq + q^2)}$$

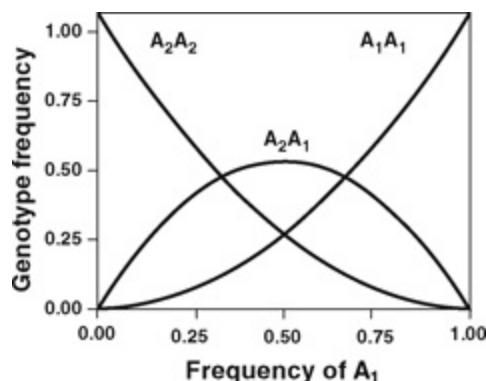
As  $p^2 + 2pq + q^2 = (p + q)^2 = 1$ , this simplifies to:

$$p_1 = p^2 + pq = p(p + q) = p$$

Likewise the frequency of A<sub>2</sub> is  $q_1 = q$ . Consequently, allele and genotype frequencies are at equilibrium after one generation of random mating, and remain so in perpetuity in the absence of other influences.

Hardy–Weinberg equilibrium is expected for all loci, except for those located on sex chromosomes (for reasons detailed below).

The relationships between allele and genotype frequencies according to the Hardy–Weinberg equilibrium are shown in Fig. 4.1. This illustrates two points. First, the frequency of heterozygotes cannot be greater than 50% for a locus with two alleles. This maximum occurs when both alleles have frequencies of 0.5. Second, when an allele is rare, it is mostly found in heterozygotes, while it is mainly in homozygotes when it is at a high frequency. For example, when the  $A_2$  allele is at a frequency of 0.1, the frequency of  $A_2A_2$  homozygotes is 0.01, and that of heterozygotes 0.18 (Table 4.3). Ninety per cent of the  $A_2$  alleles are in heterozygotes. Conversely, at a frequency of 0.5, the three genotypes have frequencies of 0.25, 0.50 and 0.25. Here half the alleles are in heterozygotes and half in homozygotes.



**Fig. 4.1** Relationship between genotype frequencies and allele frequencies in a population in Hardy–Weinberg equilibrium.

Genotype frequencies for most loci usually agree with Hardy–Weinberg expectations in large naturally outbreeding populations

To obtain the Hardy–Weinberg equilibrium we have assumed:

- a large population size
- a closed population (no migration)
- random union of gametes
- normal Mendelian segregation of alleles
- equal fertility of parent genotypes
- equal fertilizing capacity of gametes
- equal survival of all genotypes
- no mutation.

The genotype frequencies for the eider duck egg-white protein locus are tested for agreement with the Hardy–Weinberg equilibrium in [Table 4.4](#). Values of  $p$  and  $q$ , calculated previously, are used to calculate  $p^2$ ,  $2pq$  and  $q^2$ . These frequencies are then multiplied by the total number (67) to obtain expected numbers for the three genotypes.

**Table 4.4** *Test for agreement of genotype frequencies with Hardy–Weinberg equilibrium expectations for the eider duck egg-white protein locus*

	Genotypes			Total
	FF	FS	SS	
Observed numbers (O)	37	24	6	67
Expected frequencies	$p^2$	$2pq$	$q^2$	1.0
	$0.73^2$	$2 \times 0.73 \times 0.27$	$0.27^2$	1.0
	0.5329	0.3942	0.0729	1.0
Expected numbers (E) (expected frequency $\times$ 67)	35.7	26.4	4.9	67

The observed numbers for each genotype are very close to the numbers expected from the Hardy–Weinberg equilibrium. To determine if the

differences between observed and expected numbers are of statistical significance (i.e. unlikely to be due to chance alone), the deviation between observed numbers ( $O$ ) and expected numbers ( $E$ ) is tested using a  $\chi^2$  test (Box 4.1). In this case, the differences between the observed and expected numbers are not significant. In general, agreement with expectations is found for most loci in large naturally **outbreeding** populations (more or less random mating). This does not mean that the loci are free from the effects of mutation, migration, selection and sampling, only that these effects are usually too small to be detected with realistic sample sizes.

### Box 4.1 Chi-square ( $\chi^2$ ) test to assess agreement between observed and expected numbers

Differences between observed and expected numbers will occur by chance. To determine if the differences are of statistical significance, the deviation between observed numbers ( $O$ ) and expected numbers ( $E$ ) is tested using a chi-square ( $\chi^2$ ) test, computed as follows:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

where  $\Sigma$  refers to the sum of the values. The larger the difference between observed and expected, the larger the  $\chi^2$  value. The  $\chi^2$  value for the data in Table 4.4 is computed as follows:

$$\begin{aligned} \sum \frac{(O - E)^2}{E} &= \frac{(37 - 35.7)^2}{35.7} + \frac{(24 - 26.4)^2}{26.4} + \frac{(6 - 4.9)^2}{4.9} \\ \chi^2 &= 0.047 + 0.218 + 0.247 = 0.512 \\ df &= 3 - 1 - 1 = 1 \end{aligned}$$

The probability of obtaining a  $\chi^2$  of at least 0.512 purely by chance is 47% (determined using statistical software). Thus, we conclude that the observed genotype frequencies do not differ significantly from the expectations. If the probability had been  $<0.05$ , we would have concluded that there was a significant deviation from expectations.

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## Expected heterozygosity

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The Hardy–Weinberg expected heterozygosity ( $H_e$ ) is usually reported when describing genetic diversity, as it is less sensitive to sample size than observed heterozygosity

For a single locus with two alleles at frequencies of  $p$  and  $q$ , the **expected heterozygosity** ( $H_e$ ) is  $2pq$  (also called **gene diversity**), as this is the heterozygosity expected under Hardy–Weinberg equilibrium. When there are more than two alleles, it is simpler to calculate expected heterozygosity as one (the total) minus the frequencies of all homozygotes:

$$H_e = 1 - \sum_{i=1}^{\text{\#alleles}} p_i^2 \quad (4.2)$$

where  $p_i$  is the frequency of the  $i$ th allele. The reasoning behind this is that since  $p^2 + 2pq + q^2 = 1$ , then  $2pq = 1 - p^2 - q^2$ , an expression corresponding to [Equation 4.2](#).

Example 4.3 illustrates the calculation of expected heterozygosity for the microsatellite locus in Laysan finches.

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### Example 4.3 Calculating expected heterozygosity for a microsatellite locus in the Laysan finch

The allele frequencies for the 91, 95 and 97 alleles are 0.364, 0.352 and 0.284, respectively (Example 4.2). Consequently, the Hardy–Weinberg expected heterozygosity is:

$$\begin{aligned}H_e &= 1 - (0.364^2 + 0.352^2 + 0.284^2) \\&= 1 - (0.1325 + 0.1239 + 0.0807) \\&= 0.663\end{aligned}$$

The observed and expected heterozygosities of 0.659 and 0.663 at this locus are very similar, and do not differ significantly.

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Average heterozygosity over several loci is used to characterise genetic diversity in species or populations

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The evolutionary potential of a species depends on the genetic diversity in the genome. Thus, information on a single locus is unlikely to accurately depict genetic diversity for all loci in a species. For example, mammals have around 20 000 functional loci. Consequently, genetic diversity measures ( $H_o$ ,  $H_e$ ) are averaged over a random sample of many loci. These measures are demonstrated in [Box 4.2](#), where comparisons are made between levels of genetic diversity in endangered Ethiopian wolves, domestic dogs, gray wolves and coyotes, based on nine microsatellite loci.

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### **Box 4.2 Characterizing genetic diversity in the endangered Ethiopian wolf and its conservation implications (after Gottelli et al. 1994; Anonymous 2003)**

The Ethiopian wolf is one of the most endangered canids, existing in only six isolated areas of Ethiopia and having a total population of fewer than 400 individuals. Numbers are decreasing due to habitat destruction associated with agriculture, over-grazing and increasing human population pressure and to a recent outbreak of rabies. Further, in one population, the wolves coexist with domestic dogs and may hybridize with them. Dogs also compete for prey with wolves and may act as disease vectors.



Ethiopian wolf

Allele frequencies for nine microsatellite loci in two populations of Ethiopian wolves are shown below, one on the Sanetti Plateau, where there were very few dogs, and the other in Web Valley, where dogs were abundant. These results are compared with globally non-endangered canids, gray wolves, coyotes and domestic dogs.



Locus	Allele					A	$H_o$	$H_e$	$n_e$	Sample size
	1	2	3	4	5					
225	0.933	0.067				2	0.133	0.125	1.14	15
109	0.133	0.867				2	0.267	0.231	1.30	15
204	1.000					1	0.000	0.000	1	15
123	1.000					1	0.000	0.000	1	16
377	0.889	0.028	0.028	0.028	0.028	5	0.222	0.207	1.26	18
250	0.933	0.067				2	0.133	0.125	1.14	15
213	0.031	0.969				2	0.063	0.060	1.06	16
173	0.533	0.467				2	0.533	0.498	1.99	15
344		1.000				1	0.000	0.000	1	18
Means										
Ethiopian wolf										
Sanetti						2.0	0.150	0.138	1.21	16
Web						2.8	0.313	0.271	1.37	23
Domestic dogs						6.4	0.516	0.679	3.11	35
Gray wolf						4.5		0.620	2.63	18
Coyote						5.9		0.675	3.08	17

Genetic diversity is characterized using observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ , Equation 4.2), allelic diversity ( $A$ , Equation 4.3) and effective number of alleles ( $n_e$ , Equation 4.4). These are averaged over the nine loci at the bottom of the table. Several software packages available for analysing molecular genetic data are listed at the end of the chapter (Labate 2000).

From this information, three points of conservation relevance can be gleaned. First, the Ethiopian wolf populations have lower genetic diversity than the related, globally non-endangered gray wolf, coyote and domestic dog.

Second, the relatively ‘pure’ Sanetti population has less genetic diversity than the Web Valley population that coexists with domestic dogs, suggesting that there may be hybridization with dogs. This was verified when seven phenotypically abnormal Ethiopian wolves were found to contain alleles present in domestic dogs, but absent from ‘pure’ Ethiopian wolves (Chapter 7).

Third, observed and expected heterozygosities do not differ significantly in either of the Ethiopian wolf populations. Consequently, mating in the wolves is approximately random within populations.

The study also established that Ethiopian wolves are distinctly different from other canids, but related to gray wolves and coyotes (data not shown).

The management recommendations that arose from this study were:

- that feral domestic dogs be controlled to eliminate hybridization and disease spread
- that a captive breeding program be instituted immediately with genetically 'pure' Ethiopian wolf founders
- that the other Ethiopian wolf populations be surveyed, and
- that the Ethiopian wolf be recognized as a distinct species deserving conservation.

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Loss of genetic diversity can be measured by comparing heterozygosities from different time periods

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Conservation biologists are often concerned with changes in levels of genetic diversity over time, as loss of genetic diversity is an indication that the population is undergoing inbreeding and losing its evolutionary potential. Heterozygosity is often expressed as the proportion of heterozygosity retained over time, i.e.  $H_t/H_0$ , where  $H_t$  is the level of heterozygosity at generation  $t$  and  $H_0$  the level at some earlier time, referred to as time 0. For example,  $H_0$  may be the heterozygosity before a population crash, and  $H_t$  after the crash. The Mauritius kestrel passed through a single-pair bottleneck in 1974 and recovered to a size of 400–500 by 1997. Its average heterozygosity for microsatellites prior to the bottleneck (based on

genotyping of museum skins) was 0.23, but heterozygosity had dropped to 0.10 by 1997 (Groombridge *et al.* 2000). Hence,  $H_t/H_0 = 0.1/0.23 = 0.43$ . Thus, the Mauritius kestrel has lost 57% of the genetic diversity it possessed before the bottleneck.

## Allelic diversity

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Allelic diversity is also used to characterize genetic diversity

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Allelic diversity is another estimator of genetic diversity. For example, there are two alleles at the locus determining egg-white protein differences in eider ducks and three alleles at the microsatellite locus in Laysan finches. When there is more than one locus, allelic diversity ( $A$ ) is the number of alleles averaged across loci:

$$A = \frac{\text{total \# alleles over all loci}}{\text{\# loci}} \quad (4.3)$$

For example, the Sanetti population of the endangered Ethiopian wolf (Box 4.2) has a total of 18 alleles over the nine microsatellite loci surveyed, so  $A = 18/9 = 2.0$ . Estimates of allelic diversity are highly sensitive to sample size and should only be compared between samples when sizes are similar (referred to as **allelic richness**), or when they are adjusted to the same sample size by repeated re-sampling or rarefaction (Leberg 2002). Software such as FSTAT (Goudet 2002) is available to carry out adjustments for sample size.

A second measure reflecting the number of alleles is the **effective number of alleles**. This is the number of alleles needed to provide the same

heterozygosity if all alleles were equally frequent. For example, five alleles each at a frequency of 0.2 will contribute far more to heterozygosity ( $H_e = 0.8$ ) than five alleles at frequencies of 0.92, 0.02, 0.02, 0.02 and 0.02 ( $H_e = 0.152$ ). This measure is used as it is less sensitive to sample sizes and rare alleles. The effective number of alleles ( $n_e$ ) is calculated as:

$$n_e = \frac{1}{\sum p_i^2} \quad (4.4)$$

where  $p_i$  is the frequency of each allele, and the values are summed for all alleles. For example, the D9S905 microsatellite locus in chimpanzees has five alleles, but the effective number of alleles is 3.47 (Example 4.4). By contrast, the microsatellite locus in Laysan finch with three alleles, described in Example 4.3, has a  $n_e$  of 2.96, very close to the actual value, as the allele frequencies are almost equal.

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#### **Example 4.4 Effective number of alleles at the D9S905 microsatellite locus in chimpanzees**

The frequencies of the five alleles at the D9S905 microsatellite locus are 0.086, 0.186, 0.057, 0.243 and 0.429 (Table 3.3). Consequently, the effective number of alleles is:

$$n_e = \frac{1}{\sum p_i^2} = \frac{1}{(0.086^2 + 0.186^2 + 0.057^2 + 0.243^2 + 0.429^2)} = 3.47$$

Thus, the effective number of alleles at this locus is 3.47.

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## **Characterizing DNA sequence variation**

An increasing amount of data is becoming available on DNA sequence variation within populations. This is typically characterized as proportion of polymorphic nucleotide sites, nucleotide diversity or haplotype diversity (Nei 1987; Avise 2004), measures related to polymorphism and heterozygosity as described below.

The proportion of polymorphic nucleotide sites ( $p_n$ ) is simply the proportion of sites sequenced that are polymorphic. For example, for the *Adh* sequence variation in fruit flies (Fig. 3.2), there were 43 polymorphic sites over 2379 nucleotides sequenced, yielding  $p_n = 43/2379 = 0.0181$ .

In a random mating population, **nucleotide diversity** ( $\pi$ ) is heterozygosity at the nucleotide level. It is estimated as:

$$\pi = \frac{\sum \pi_{ij}}{n_c} \quad (4.5)$$

where  $\pi_{ij}$  is the proportion of different nucleotides between the  $i$ th and the  $j$ th sequence. This is summed over all pairwise comparisons and divided by the number of comparisons  $n_c$  (for  $n$  sequences there are  $n(n - 1)/2$  comparisons). For the F allele at the *Adh* locus in fruit flies, 5 alleles were sequenced and the  $(5 \times 4)/2 = 10$  comparisons between these yielded proportions of different nucleotides of 0, 0, 0, 0.0038, 0.0038, 0.0038, 0.0042, 0.0042, 0.0042 and 0.0046. Nucleotide diversity is the average of these values =  $0.0286/10 = 0.00286$ . Nucleotide diversity within a population often falls in the range of 0.005–0.020 (Stephan & Langley 1992), but does depend upon the locus and species examined.

**Haplotype diversity** ( $h$ ) is a DNA level analogue of heterozygosity, but for haplotypes. It is measured as  $h = 1 - \sum f_i^2$ , where  $f_i$  is the frequency of the  $i$ th haplotype. For the *Adh* locus in fruit flies, 11 haplotypes were sequenced, yielding nine different types, eight with frequencies of  $1/11$ , and one with a frequency of  $3/11$ . Thus, the haplotype diversity is:

$$h = 1 - \sum f_i^2 = 1 - [8 \times (1/11)^2 + (3/11)^2] = 1 - 0.14 = 0.86$$

## Estimating the allele frequency for a recessive allele

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The Hardy–Weinberg equilibrium provides a means for estimating the frequencies of recessive alleles in random mating populations

It is not possible to determine the frequency of an allele at a locus showing dominance (e.g. recessive deleterious conditions, AFLP and RAPD loci) using the allele counting method outlined above, as homozygotes cannot be distinguished phenotypically from heterozygotes. However, we can estimate the frequencies of recessive alleles by equating the observed frequency of homozygous recessive phenotypes to  $q^2$  (the Hardy–Weinberg equilibrium frequency for the recessive homozygote), and obtain the recessive allele frequency as the square root of this frequency. The calculation is illustrated for chondrodystrophic dwarfism in the endangered California condor in Example 4.5. This allele has an estimated frequency of 0.17, a surprisingly high frequency for a recessive lethal allele. Relatively high frequencies of particular recessive inherited defects have also been found in other populations derived from few founders, including other endangered species and genetically isolated human populations ([Chapter 7](#)).

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### **Example 4.5 Estimating the frequency of the recessive chondrodystrophy allele in California condors using the Hardy–Weinberg equilibrium frequencies**

Chondrodystrophy in California condors is a condition that results in shortening in the long bones (dwarfing) and death around hatching. It is

thought to be due to homozygosity for a recessive allele, as are similar conditions in domestic turkeys. Of 169 hatched eggs in the condors, five exhibited chondrodystrophy, a frequency of 0.0296 (Ralls *et al.* 2000). If we use + and *dw* as the symbol for the normal and chondrodystrophic alleles, and *p* and *q* for their frequencies, the phenotypes, corresponding genotypes, and expected Hardy–Weinberg equilibrium frequencies are as follows:

	Phenotypes	
	Normal	Chondrodystrophic
Genotypes	(++ and + <i>dw</i> )	<i>dw</i> <i>dw</i>
Observed frequency	0.9704	0.0296
H–W equilibrium expected frequency	$p^2 + 2pq$	$q^2$



California condor

If the assumptions of the Hardy–Weinberg equilibrium are upheld, we can estimate the frequency of the *dw* allele by equating the observed frequency of affected individuals to  $q^2$  and solving, as follows:

$$q^2 = 0.0296$$

and

$$q = \sqrt{q^2} = \sqrt{0.0296} = 0.17$$

The chondrodystrophy allele has a frequency of about 17% at hatching. This is a very high level for a deleterious allele, but is not surprising given that the condors have been reduced to very low numbers (minimum of 14 individuals).

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Since there are several assumptions underlying this method of estimating  $q$  (random mating, no selection or migration), it should never be used for loci where all genotypes can be distinguished.

## Frequency of carriers (heterozygotes)

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The Hardy–Weinberg equilibrium allows us to predict the frequency of carriers for genetic diseases

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The frequency of carriers of recessive mutations is of interest in conservation genetics, as well as in human and veterinary medicine. However, carriers of deleterious recessives ( $Aa$ ) cannot be distinguished from non-carriers ( $AA$ ). Nevertheless, we can predict the frequency of carriers amongst those with normal phenotypes from the Hardy–Weinberg equilibrium as the ratio of the frequency of heterozygotes ( $Aa = 2pq$ ) to that of all individuals with normal phenotypes ( $AA + Aa = p^2 + 2pq$ ), which upon dividing both by  $p$  and substituting  $p + q = 1$  simplifies to:

$$\text{frequency (carriers)} = \frac{2q}{(1 + q)} \quad (4.6)$$

In the case of chondrodystrophy, the frequency of carriers among individuals with normal phenotypes is expected to be  $2 \times 0.17 / (1 + 0.17) = 0.29$ . Thus, about 30% of the condor population are carriers, almost 10 times the frequency of affected homozygotes (if we can assume random mating).



## Deviations from Hardy–Weinberg equilibrium

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Deviations from Hardy–Weinberg equilibrium genotype frequencies are highly informative, allowing us to detect inbreeding, population fragmentation, migration and selection

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The Hardy–Weinberg equilibrium provides a null hypothesis against which to test whether the population has non-random mating, migration, or selection. Put in another way, deviations from Hardy–Weinberg equilibrium indicate that one or more of the assumptions we listed earlier is being violated. We deal with non-random mating below while selection is covered in [Chapter 6](#) and migration in [Chapter 7](#).

### Inbreeding

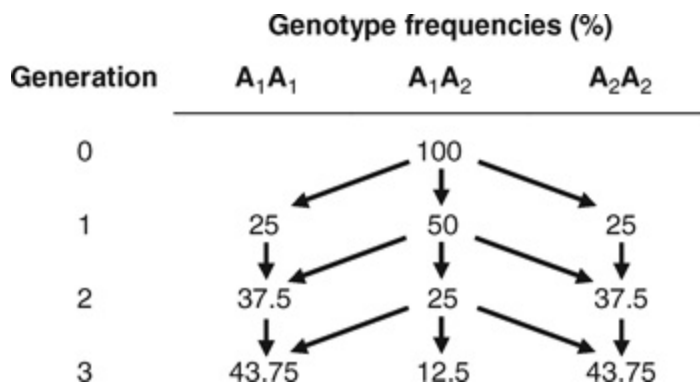
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Inbreeding reduces the frequency of heterozygotes compared to random mating

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Inbreeding is of major importance in conservation genetics as it leads to reduced reproductive fitness ([Chapters 2, 12 and 13](#)). When related individuals mate at a rate greater than expected by random mating, the frequency of heterozygotes is reduced relative to Hardy–Weinberg expectations, and homozygote frequencies are correspondingly increased.

This is illustrated for self-fertilization (the most extreme form of inbreeding) in Fig. 4.2. Selfing an  $A_1A_2$  individual results in halved heterozygosity in the progeny. By generation 2, the frequency of heterozygotes is 25%, compared to the Hardy–Weinberg equilibrium expectation of 50%, and it continues to halve in each subsequent generation.



**Fig. 4.2** Effect of self-fertilization on genotype frequencies. *The frequency of heterozygotes halves with each generation of selfing.*

Consequently, deficiencies of heterozygotes in populations, compared to Hardy–Weinberg equilibrium expectations, indicate that they are not mating randomly, as illustrated for phlox plants in Table 4.5. This species shows a high level of self-fertilization. In contrast, while inbreeding leads to lower than expected heterozygosity, outcrossing and avoidance of inbreeding can lead to higher than expected heterozygosity. Inbreeding is treated in detail in Chapter 12.

**Table 4.5** *Heterozygote deficiency in an inbreeding plant population. Observed numbers for the three genotypes at the phosphoglucomutase-2 locus in a phlox population are given, along with expected numbers for a random-mating population in Hardy–Weinberg equilibrium. In this species, 78% of the seeds are estimated to result from self-fertilization*

	Genotypes		
	FF	FS	SS
Observed numbers	15	6	14
Hardy–Weinberg expectations	9.3	17.5	8.3

*Source: Data from Hartl & Clark (2007) after Levin.*

## **Fragmented populations**

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Fragmented populations with restricted gene flow show deficiencies of heterozygotes compared to Hardy–Weinberg expectations

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Allele frequencies diverge among isolated populations due to chance and selection ([Chapter 14](#)). This results in an overall deficiency of heterozygotes, even when individual populations are themselves in Hardy–Weinberg equilibrium. In the extreme case, where one population has only allele  $A_1$  and the other has only allele  $A_2$ , there are no heterozygotes. This is much less than the overall random mating expectation of 50% heterozygosity with two alleles at frequencies of 0.5. For example, two isolated populations of black-footed rock wallabies on Barrow and Mondrain Islands off the Western Australian coast are homozygous for alleles 136 and 124, respectively, at the Pa297 microsatellite locus ([Table 14.1](#)).

Heterozygote deficiency can also be used to identify cryptic species – species that are morphologically so similar they are difficult to tell apart without molecular techniques. For example, when velvet worms (see chapter frontispiece) from a single log in the Blue Mountains near Sydney were genotyped using allozyme electrophoresis, genetic diversity was detected at several loci, but there were no heterozygotes. Two forms that differed slightly in body colour were both found to be homozygous at all loci, but they were homozygous for different alleles at 86% of the sampled loci. This was a clear indication that the two forms (previously considered to belong to a single species) were not exchanging alleles, even though they shared the same habitat. Consequently, this led to them being designated as separate species

(Briscoe & Tait 1995). Genetic markers can be used to determine whether there is gene flow between populations, and so assist in resolving taxonomic uncertainties (Chapter 16).

## Extensions of the Hardy–Weinberg equilibrium

### Three alleles

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Hardy–Weinberg equilibrium occurs for autosomal loci with any number of alleles in random mating populations

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Expressions for the Hardy–Weinberg equilibrium can be obtained for more than two alleles at a locus. If there are three alleles  $A_1$ ,  $A_2$  and  $A_3$  at a locus with frequencies  $p$ ,  $q$  and  $r$ , then the Hardy–Weinberg equilibrium genotype frequencies are given by the terms of the binomial expansion  $(p + q + r)^2$  (Example 4.6). The genotype frequencies can be derived in a similar manner to those for a diallelic locus (Table 4.3). Example 4.6 illustrates calculation of expected genotype frequencies for a locus with three alleles in the endangered Laysan finch.

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#### **Example 4.6 Calculating the expected Hardy–Weinberg equilibrium frequencies at a locus with three alleles**

For the Laysan finches the frequencies of the 91, 95 and 97 microsatellite alleles are 0.364 ( $p$ ), 0.352 ( $q$ ) and 0.284 ( $r$ ), respectively (Table 4.2). Consequently, the expected genotype frequencies are:

	Genotypes						Total
	91/91	91/95	91/97	95/95	95/97	97/97	
Expected	$p^2$	$2pq$	$2pr$	$q^2$	$2qr$	$r^2$	1.0
frequencies	$0.364^2$	$2 \times 0.364 \times 0.352$	$2 \times 0.364 \times 0.284$	$0.352^2$	$2 \times 0.352 \times 0.284$	$0.284^2$	1.0
	0.132	0.256	0.207	0.124	0.200	0.081	1.0

## Sex-linked loci

As sex-linked loci exist in different numbers of copies in females and males, they have different genotype frequencies in the two sexes

In mammals and fruit flies, **sex-linked loci** are located on the X chromosome, with females having XX sex chromosomes and males XY. The Y chromosome lacks most of the loci present on the X. Thus females have two copies of most loci and males have only one. Conversely, birds and Lepidoptera have ZZ males and ZW females, with two copies of sex-linked loci in males and one in females. When dealing with sex-linked loci we will display alleles as superscripts on the X or Z chromosomes (e.g.  $X^A$ , or  $Z^A$ ) and the Y or W as devoid of sex-linked alleles, to avoid confusion with autosomal loci.

It is important to distinguish sex-linked loci as they have Hardy–Weinberg equilibrium genotype frequencies that differ from those for autosomal loci. Consequently, these differences could be confused with the effects of inbreeding, non-random mating, or population fragmentation. In mammals 2/3 of the sex-linked alleles are found in females and 1/3 in males when there is an equal sex-ratio, while these proportions in the two sexes are reversed in

birds and Lepidoptera.

The procedure for calculating allele frequencies for a sex-linked locus is similar to the allele counting method used in Example 4.1, except that we must take account of the different number of copies of loci in females and males. Table 4.6 illustrates genotype frequencies for the sex-linked 6-pgd locus in a *Heliconius* butterfly from Trinidad, and Example 4.7 illustrates the estimation of allele frequencies.

**Table 4.6** *Numbers of each genotype in females and males at the sex-linked 6-phosphogluconate dehydrogenase enzyme (6-pgd) locus in a Heliconius butterfly from Trinidad, and the numbers expected with Hardy–Weinberg equilibrium. Note that males have ZZ and females ZW sex chromosomes*

	Males				Females		
	$Z^F Z^F$	$Z^F Z^S$	$Z^S Z^S$	Total	$Z^F W$	$Z^S W$	Total
Numbers	39	46	27	112	29	33	62
Expected	32.1	55.7	24.2	112	33.2	28.8	62

Source: Simplified from Johnson & Turner (1979).



Heliconius butterfly

### **Example 4.7 Estimation of allele frequencies at the sex-linked 6-pgd locus in Heliconius butterflies from Trinidad**

The frequency of the  $Z^F$  allele is obtained by counting the number of copies of the F allele in all the females and males, as done previously.

However, for a sex-linked locus in butterflies, females have one copy of the locus and males two. Thus, the number of F alleles is  $2 \times 39 = 78$  from the  $Z^F Z^F$  males, 46 from the  $Z^F Z^S$  males and 29 from the  $Z^F W$  females, totalling 153 F alleles.

We divide this by the total number of allele copies in the sample, counting two for each male ( $2 \times 112$ ) and one for each female 62, totalling 286.

Thus, the frequency of the sex-linked  $Z^F$  allele ( $p$ ) is:

$$p = \frac{[(2 \times 39) + 46 + 29]}{[(2 \times 112) + 62]} = \frac{153}{286} = 0.535$$

Similarly, the frequency of the sex-linked  $Z^S$  allele ( $q$ ) is:

$$q = \frac{[(2 \times 27) + 46 + 33]}{[(2 \times 112) + 62]} = \frac{133}{286} = 0.465$$

and checking  $p + q = 0.535 + 0.465 = 1$ .

Thus, the frequency of the sex-linked F allele is 53.5% and that of S is 46.5%.

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Sex-linked loci reach Hardy–Weinberg equilibrium under random mating with different genotype frequencies in females and males

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For sex-linked loci at Hardy–Weinberg equilibrium in species with XX :

XY sex-chromosomes, female genotype frequencies are the same as those for autosomal loci, while male genotype frequencies are  $p$  and  $q$ , the allele frequencies. If the allele frequencies in males and females are equal, the population reaches equilibrium allele and genotype frequencies in one generation with random mating (and no other perturbing forces), as for autosomal loci (Table 4.7). In contrast, populations with different allelic frequencies in the two sexes only approach equilibrium genotype frequencies asymptotically over generations, with equilibrium attained when allele frequencies are equal in females and males (Falconer & Mackay 1996).

**Table 4.7** *Hardy–Weinberg equilibrium genotype frequencies in females and males for a sex-linked locus following random mating in a species with XX females and XY males*

Females			Males	
$X^A X^A$	$X^A X^a$	$X^a X^a$	$X^A Y$	$X^a Y$
$p^2$	$2pq$	$q^2$	$p$	$q$

For birds and Lepidoptera with ZZ males and ZW females, the males and females are reversed from those in Table 4.7. Observed and Hardy–Weinberg expected numbers for the sex-linked 6-pgd locus in the *Heliconius* butterfly population are compared in Table 4.6. Neither the male nor the female numbers deviate significantly from Hardy–Weinberg expectations, but there is a suspicious deficiency of heterozygotes in males that could be due to combining individuals from partially isolated populations.

## Polyploids

Hardy–Weinberg equilibrium occurs in tetraploids, but results in a higher frequency of heterozygotes than found in diploids when allele frequencies are the same



Many plants and a small number of animals have more than two copies of each chromosome (**polyploids**). For example, some populations of the endangered grassland daisy in south-eastern Australia have four copies of each chromosome (tetraploid) rather than two (Young & Murray 2000) (see Chapter 16).

In what follows, we consider only tetraploids, but the same principles apply to other even-number ploidies ( $6n$  hexaploid,  $8n$  octoploid, etc.). Triploids ( $3n$ ) typically have abnormal meiosis and produce few viable seeds.

Allele frequencies in tetraploids are calculated by the same allele counting method used in diploids, but there are four copies of each locus in each individual, and five genotypes for a locus with two alleles (Table 4.8). In an autotetraploid, Hardy–Weinberg equilibrium genotype frequencies at a locus with two alleles are given as the terms of the binomial expansion  $(p + q)^4$  (Table 4.8).

**Table 4.8** *Hardy–Weinberg equilibrium genotype frequencies in a random mating autotetraploid*

	Genotypes					Total
	$A_1A_1A_1A_1$	$A_1A_1A_1A_2$	$A_1A_1A_2A_2$	$A_1A_2A_2A_2$	$A_2A_2A_2A_2$	
Frequency	$p^4$	$4p^3q$	$6p^2q^2$	$4pq^3$	$q^4$	1.0
Example: $p = 0.6$ and $q = 0.4$						
Frequency	0.1296	0.3456	0.3456	0.1536	0.0256	1.0

At a polymorphic locus with two alleles in a tetraploid, there are three heterozygous genotypes ( $A_1A_1A_1A_2$ ,  $A_1A_1A_2A_2$  and  $A_1A_2A_2A_2$ ) with a total frequency of:

$$\begin{aligned}
 H_e &= 4p^3q + 6p^2q^2 + 4pq^3 = 2pq (2p^2 + 3pq + 2q^2) \\
 &= 2pq (2 - pq)
 \end{aligned}$$

(4.7)

Thus, the frequency of heterozygotes of  $2pq$  ( $2 - pq$ ) is considerably greater than the  $2pq$  frequency for an equivalent diploid (Bever & Felber 1994). For example, if we have two alleles with frequencies of 0.6 and 0.4, the frequency of heterozygotes in an autotetraploid is 0.84, compared to 0.48 in an equivalent diploid. This has important implications when we consider loss of genetic diversity and inbreeding in small populations (Chapters 11–13), as well as for genetic management of polyploids (Chapter 17).

## More than one locus: linkage disequilibrium

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Alleles at different loci are expected to be randomly associated in a large random mating population at equilibrium, i.e. they show linkage equilibrium

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Consider two loci, A and B with alleles  $A_1$ ,  $A_2$  and  $B_1$ ,  $B_2$  and frequencies  $p_A$ ,  $q_A$ ,  $p_B$ ,  $q_B$  respectively. These alleles form gametes  $A_1B_1$ ,  $A_1B_2$ ,  $A_2B_1$  and  $A_2B_2$ . Under random mating and independent assortment, these gametes will have frequencies that are the product of their component allele frequencies. For example, gamete  $A_1B_2$  will have a frequency of  $p_A q_B$ . Random association of alleles at different loci is referred to as **linkage equilibrium**. Alleles at most loci in large random mating populations are in linkage equilibrium unless they are very closely linked (Ardlie *et al.* 2002).

---

Linkage disequilibrium describes the conditions where there is a deviation from random combinations of allele frequencies at different loci, such that the fate of an allele will be correlated with that of neighbouring loci

Chance events in small populations, population bottlenecks, recent mixing of different populations and selection all may cause non-random associations among loci, particularly where the loci are physically linked on the same chromosome ([Box 4.3](#)). Loci that show such **linkage disequilibrium** in large random mating populations are often subject to strong forces of natural selection (see [Box 4.4](#) below).

### **Box 4.3 Effects of linkage disequilibrium**

To demonstrate the effects of linkage disequilibrium, let us consider an example where two different monomorphic populations with genotypes  $A_1A_1B_1B_1$  and  $A_2A_2B_2B_2$ , are combined and allowed to mate at random. Each autosomal locus is expected to attain individual Hardy–Weinberg equilibrium within one generation of random mating (see above). However, alleles at different loci only approach linkage equilibrium asymptotically at a rate dependent on the recombination frequency between the two loci. In the above-pooled population, let 70% have genotype  $A_1A_1B_1B_1$  and 30%  $A_2A_2B_2B_2$  with equal numbers of males and females for each of the two genotypes. Only two gametes are produced,  $A_1B_1$  and  $A_2B_2$ , so progeny in the next generation will consist of only three genotypes  $A_1A_1B_1B_1$ ,  $A_1A_2B_1B_2$  and  $A_2A_2B_2B_2$ , and none of the other six possible genotypes. These loci are clearly in linkage disequilibrium.

In subsequent generations, the two other possible gametes  $A_1B_2$  and  $A_2B_1$  are generated by recombination in the doubly heterozygous genotype. For example,  $A_1B_1/A_2B_2$  heterozygotes produce recombinant gametes  $A_1B_2$  and  $A_2B_1$  at frequencies of  $\frac{1}{2}c$ , where  $c$  is the rate of recombination, as well as non-recombinant  $A_1B_1$  and  $A_2B_2$  gametes in frequencies of  $\frac{1}{2}(1 - c)$ . If the two loci are located on different chromosome pairs, they will show independent assortment,  $c = 0.5$ . If the loci are physically linked,  $c \leq 0.5$  and it will generally take longer to achieve linkage equilibrium. Eventually all nine possible genotypes will be formed and attain linkage equilibrium.

---

---

#### **Box 4.4 Linkage disequilibrium at the major histocompatibility complex (MHC) in humans**

The data below show non-random associations between alleles at the HLA-A and HLA-B loci in the MHC of Caucasians based on 2106 haplotypes (data after Spiess [1989](#)). The data have been simplified to show only four alleles at one locus and three at the other. There are actually many more alleles (for this reason, the total of allele frequencies for each locus in our example do not add to unity).

All of the non-random associations between alleles at the two loci, as shown by the sign of the deviation from expectation, are statistically significant. For example, the frequency of the A1-B7 haplotype was 0.0074. At linkage equilibrium it would be expected to have a frequency of  $0.1439 \times 0.1143 = 0.0164$ , so that it shows a deficiency of  $-0.0090$ . This deficiency is the linkage disequilibrium associated with this haplotype and is 55% of the maximum value that  $D$  could have for alleles with these frequencies.

Haplotype frequencies for HLA-A and HLA-B loci. The sign after each figure indicates a deficiency (-), or excess of (+) of the haplotype

		HLA-A alleles			Overall HLA-B allele frequencies
		A1	A2	A3	
HLA-B allele	B7	0.0074(-)	0.0260(-)	0.0477(+)	0.1143
	B8	0.0672(+)	0.0110(-)	0.0019(-)	0.0971
	B35	0.0029(-)	0.0178(-)	0.0257(+)	0.1052
	B44	0.0089(-)	0.0503(+)	0.0068(-)	0.1242
Overall HLA-A allele frequencies		0.1439	0.2855	0.1335	

In small populations, neutral alleles that have no selective difference between genotypes may behave as if they are under selection, due to non-random association with alleles at nearby loci that are being strongly selected.

Linkage disequilibrium is of importance in populations of conservation concern, because:

- linkage disequilibrium will be common in threatened species as their population sizes are small (Service *et al.* 2006)
- evolutionary processes are altered when there is linkage disequilibrium
- functionally important gene clusters exhibiting linkage disequilibrium (such as MHC and self-incompatibility loci) are of major importance to the persistence of threatened species
- linkage disequilibrium is one of the signals that can be used to detect admixture of differentiated populations
- linkage disequilibrium can be used to estimate genetically effective population sizes (Chapter 11).

The effects of linkage disequilibrium are illustrated in Box 4.3 via an example.

Linkage disequilibrium is measured as the deviation of haplotype frequencies from linkage equilibrium

As shown in Table 4.9, the measure of linkage disequilibrium  $D$  is the difference between the product of the frequencies of the  $A_1B_1$  and  $A_2B_2$  gametes (referred to as  $r$  and  $u$ ) and the product of the frequencies of the  $A_1B_2$  and  $A_2B_1$  gametes ( $s$  and  $t$ ):

$$D = ru - st \quad (4.8)$$

**Table 4.9** *Measuring linkage disequilibrium among alleles at two loci, A and B. Each locus has two alleles  $A_1$ ,  $A_2$  and  $B_1$ ,  $B_2$  at frequencies  $p_A$ ,  $q_A$  and  $p_B$ ,  $q_B$ , respectively*

	Gametic types (haplotypes)				Total
	$A_1B_1$	$A_1B_2$	$A_2B_1$	$A_2B_2$	
Actual frequencies	$r$	$s$	$t$	$u$	1.0
Equilibrium frequencies	$p_A p_B$	$p_A q_B$	$q_A p_B$	$q_A q_B$	1.0
Disequilibrium $D = ru - st$					
Numerical example: $p_A = 0.7$ , $q_A = 0.3$ , $p_B = 0.7$ , $q_B = 0.3$					
Actual frequencies	0.7	0.0	0.0	0.3	
Equilibrium frequencies	$0.7 \times 0.7$ = 0.49	$0.7 \times 0.3$ = 0.21	$0.3 \times 0.7$ = 0.21	$0.3 \times 0.3$ = 0.09	
$D = 0.7 \times 0.3 - 0.0 \times 0.0 = 0.21$					

Note that under equilibrium  $ru = st$ , since both  $ru$  and  $st$  are equal to  $p_A q_A p_B q_B$ , and  $D = 0$ . In our example, the four gametic types had frequencies of 0.7, 0, 0, and 0.3 in the first generation, so  $D = 0.7 \times 0.3 - 0 \times 0 = 0.21$ . The maximum value of  $D$  is 0.25, and the minimum -0.25. The maximum

value of  $D$  occurs when the frequencies of the four gametic types are  $\frac{1}{2}$ , 0, 0 and  $\frac{1}{2}$ .

In general, loci further apart than 1 centimorgan (1% crossing-over) in large random mating populations do not show linkage disequilibrium unless they involve a cluster of loci that are subject to balancing selection ([Chapter 9](#)). Linkage disequilibrium is found in the major histocompatibility complex (MHC), a cluster of loci involved in immune response, transplant rejection and fighting disease organisms in vertebrates ([Chapter 9](#)) ([Box 4.4](#)). As expected, linkage disequilibrium between pairs of loci in the MHC is generally greater for loci that are closer to each other (Hedrick *et al.* [1991](#)).

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Linkage disequilibrium decays at a rate dependent on the recombination rate between the loci

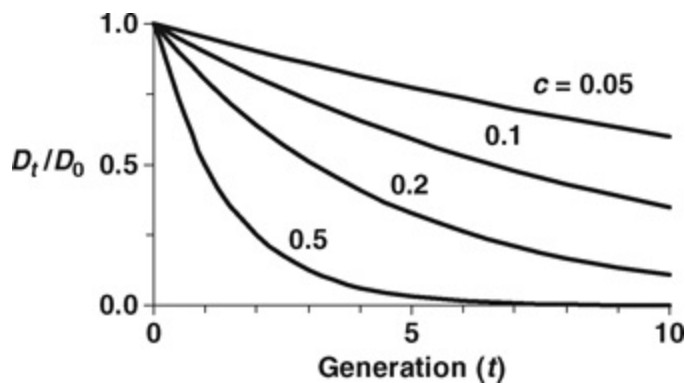
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Linkage disequilibrium decays as recombination produces underrepresented gametes. Recombination results from independent segregation of unlinked loci and crossing-over between linked loci. In our first example above, linkage disequilibrium decayed with random mating as  $A_1B_2$  and  $A_2B_1$  gametes were produced by recombination in multiply heterozygous genotypes. The rate of decay of disequilibrium depends on the recombination frequency as shown in [Fig. 4.3](#). After  $t$  generations, the remaining disequilibrium is (Falconer & Mackay [1996](#)):

$$D_t = D_0(1 - c)^t \tag{4.9}$$

where  $c$  is the recombination frequency. The amount of recombination between two loci depends on their positions on a chromosome, generally

increasing with the distance between loci. Linkage disequilibrium halves each generation for unlinked loci, with approximate linkage equilibrium reached in five generations. Conversely, decay of disequilibrium is slow for closely linked loci.



**Fig. 4.3** Decay in linkage disequilibrium between two loci under random mating, with recombination frequencies of  $c = 0.05$  0.1, 0.2 and 0.5 (unlinked loci). The proportion of linkage disequilibrium remaining ( $D_t/D_0$ ) is plotted against generations ( $t$ ).

## Summary

1. Genetic diversity within a population is characterized by the frequencies of each of the genotypes. This is normally simplified by reporting the allele (gene) frequencies at each locus.
2. In large random mating populations with no perturbing factors, the allele and genotype frequencies at autosomal loci are in equilibrium after one generation (Hardy–Weinberg equilibrium).
3. Deviations from Hardy–Weinberg equilibrium genotype frequencies allow us to detect inbreeding, population fragmentation, migration and selection.
4. A deficit of heterozygotes often indicates that inbreeding is occurring at a higher than expected rate, or that the population is fragmented.
5. If there are non-random associations between alleles at different loci (linkage disequilibrium), the fate of an allele will be affected by that of neighbouring loci. Linkage disequilibrium decays towards equilibrium at a rate dependent on recombination frequency between



the loci.

## Further reading

Balding *et al.* (eds.) (2001) *Handbook of Statistical Genetics*. Advanced reviews on a broad range of topics in statistical genetics.

Falconer & Mackay (1996) *Introduction to Quantitative Genetics*. Contains a very clear introduction to topics in this Chapter.

Hartl & Clark (2007) *Principles of Population Genetics*. Clear introduction to population genetics.

Hedrick (2005a) *Genetics of Populations*. Clearly written coverage of population genetics.

## Software

The following software packages can be used for analysing molecular population genetics data:

ARLEQUIN: [www.anthropologie.unige.ch/arlequin](http://www.anthropologie.unige.ch/arlequin)

FSTAT: [www2.unil.ch/popgen/softwares/fstat.htm](http://www2.unil.ch/popgen/softwares/fstat.htm)

GDA: GENETIC DATA ANALYSIS:  
[www.lewis.eeb.uconn.edu/lewishome/gda.html](http://www.lewis.eeb.uconn.edu/lewishome/gda.html)

GENALEX: [www.anu.edu.au/BoZo/GenALEX/](http://www.anu.edu.au/BoZo/GenALEX/)

GENEPOP (Rousset 2008): <http://kimura.univ-montp2.fr/?rousset/Genepop.htm>

POPGENE: [www.ualberta.ca/~fyeh/index.html](http://www.ualberta.ca/~fyeh/index.html)

## Problems

**4.1** Allele frequency. Estimate the frequencies for the M and N alleles in the following sample of humans from Greenland (after Falconer & Mackay 1996).

	Blood group genotype			
	MM	MN	NN	Total
Numbers	475	89	5	569

- 4.2 Hardy–Weinberg equilibrium. What are the expected Hardy–Weinberg equilibrium frequencies and numbers at the MN blood group locus in the human population described in Problem 4.1? Do the observed numbers differ from those expected according to the Hardy–Weinberg equilibrium?
- 4.3 Allele frequencies for a locus with four alleles. What are the frequencies of the 85, 91, 93 and 95 alleles at the 11B4E microsatellite locus in the endangered Laysan finch of Hawaii (data of Tarr *et al.* 1998)? Check that the allele frequencies add to unity.

	Genotypes									
	85/85	85/91	85/93	85/95	91/91	91/93	91/95	93/93	93/95	95/95
Numbers	2	13	0	0	15	2	12	0	0	0

- 4.4 Observed heterozygosity. What is the observed heterozygosity for the microsatellite locus in Laysan finches in Problem 4.3?
- 4.5 Hardy–Weinberg equilibrium for a triallelic locus. What are the expected genotypic frequencies for the microsatellite locus in Laysan finches from Table 4.2? What are the expected numbers? Do the observed numbers agree with Hardy–Weinberg expectations?
- 4.6 Hardy–Weinberg equilibrium in trisomics. If the frequencies of the F and S alleles at an electrophoretic locus situated on chromosome 21 in humans are 0.6 and 0.4, what are the Hardy–Weinberg equilibrium genotype frequencies in a population of Down's syndrome sufferers (with three doses of chromosome 21)?
- 4.7 Random mating. The three alleles at the human ABO blood group have frequencies of about 0.3 A, 0.1 B and 0.6 O in Caucasians. What is the expected frequency of AA × OO matings?
- 4.8 Random mating. Is the human population in Ashibetsu, Japan, mating at random with respect to the MN blood group locus? The number of people with each of the blood group genotypes were (from Strickberger 1985 after Matsunaga & Itoh):

MM	MN	NN	Total
406	744	332	1482

and the 741 mating couples had the following distribution of genotyp

MM × MM	58
MM × MN	202
MM × NN	88
MN × MN	190
MN × NN	162
NN × NN	41

- 4.9** Effective number of alleles. What is the effective number of alleles for the eider duck egg-white protein locus described in [Table 4.1](#)?
- 4.10** Characterizing DNA sequence diversity. For the S *Adh* allele sequence data in *Drosophila melanogaster* (Kreitman [1983](#)), compute proportion of polymorphic nucleotide sites, nucleotide diversity and haplotype diversity, given that the six S alleles were sequenced for 2379 nucleotides with 30 sites polymorphic, that all six haplotypes were different from each other and that the proportion of nucleotides different among the six haplotypes were 0.0013, 0.0059, 0.0055, 0.0067, 0.0063, 0.0025, 0.0080, 0.0084, 0.0055, 0.0046, 0.0080, 0.0067, 0.0038, 0.0046 and 0.0059 (Nei [1987](#)).
- 4.11** Allele frequency for a recessive. Assume that hernias in golden lion tamarins are inherited as an autosomal recessive and that 96 individuals have the normal phenotype and four have hernias. What is the frequency of the recessive allele causing hernias?
- 4.12** Linkage disequilibrium. What is the linkage disequilibrium  $D$  in a population with the following gametic frequencies? What will be the gametic frequencies at equilibrium?

	$A_1B_1$	$A_1B_2$	$A_2B_1$	$A_2B_2$
Frequency	0.2	0.5	0.2	0.1

- 4.13** Decay of linkage disequilibrium. If the linkage disequilibrium between two loci  $D$  is 0.20 initially, and the two loci show 5% recombination, what will be the value of  $D$  after 20 generations?

## Chapter 5 Characterizing genetic diversity: quantitative variation

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Characters of concern in conservation biology are primarily quantitative, where variation is due to both genetic and environmental factors. Components of quantitative genetic variation determine a species' ability to undergo adaptive evolution, and the effects of inbreeding and outbreeding on reproductive fitness

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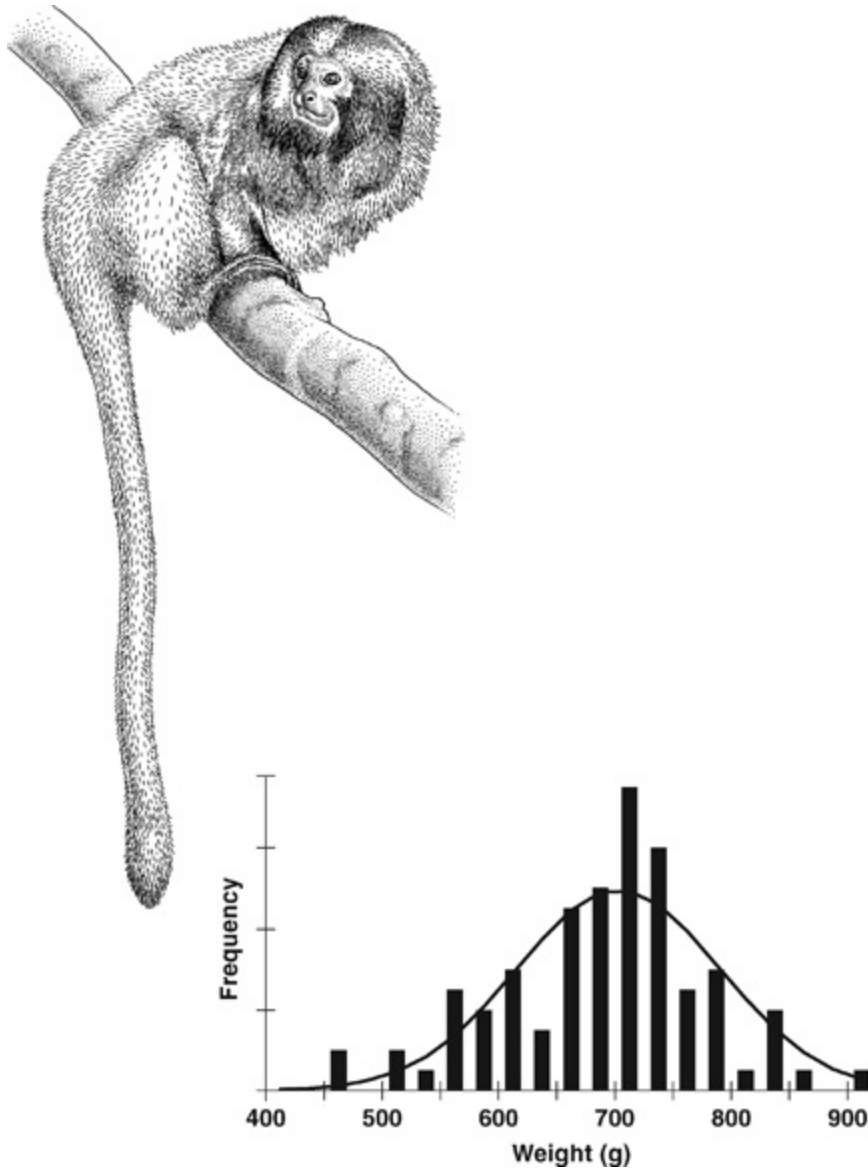
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### Terms

Additive, additive variance, common garden experiment, dominance variance, epistatic variance, genotype  $\times$  environment interaction, heritability, heterosis, interaction variance, maternal effects, normal distribution, overdominance, peripheral character, quantitative genetic variation, quantitative trait locus (QTL), reproductive fitness, selection differential, variance

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Distribution of adult body weights (a quantitative character) in golden lion tamarins

## Importance of quantitative characters

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The characters of greatest concern in conservation biology show quantitative variation among individuals

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The quantitative (or metric, or polygenic) characters of most concern to conservation biologists are those related to reproductive fitness. This is the number of fertile offspring contributed by an individual that survive to reproductive age. Quantitative variation for reproductive fitness is involved in (Hard [1995](#); Frankham [1999](#)):

- reduction in reproductive fitness due to inbreeding (inbreeding depression),
- reduced ability to evolve due to small population sizes,
- impact of crossing between different populations on fitness, whether beneficial (**heterosis**), or deleterious (**outbreeding depression**), and
- effects of translocating individuals from one environment to another.

Quantitative genetics is the study of such variation. [Box 5.1](#) illustrates quantitative variation for resistance to an introduced root rot fungus in an Australian eucalypt tree, and its conservation implications.

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**Box 5.1 Quantitative genetic variation in resistance to an introduced root-rot fungus in a Western Australian eucalypt tree and its conservation implications (Stukely & Crane [1994](#); McComb [2002](#))**

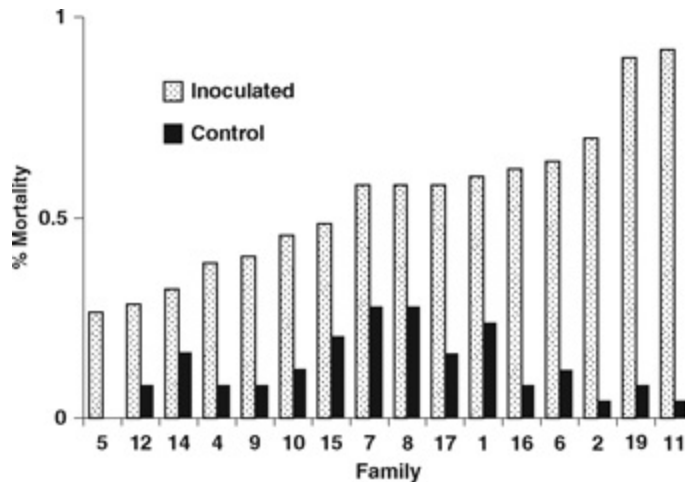
Introduced ‘dieback’ root-rot fungus is known to attack 90 Western

Australian native plant species, many rare or endangered. It has caused serious degradation to many highly diverse plant communities in the southwest of the State, where tourism to view wildflowers is a major industry. One victim is jarrah, a hardwood eucalypt tree of economic importance. Areas of jarrah forest that suffered dieback have previously been replanted with exotics, such as pines and eastern Australian eucalypts (which are relatively resistant to dieback) with subsequent alteration of habitat for native birds, mammals and invertebrates. To avoid such habitat loss, the current objective is to revegetate with native species.



Jarrah

Occasional healthy jarrah trees persist in dieback-affected sites, but it was not known whether these had fortuitously escaped infection, or whether they were genetically resistant to the disease. Seedlings of 16 families were either inoculated with the fungus, or kept as uninfected controls in an adjacent, disease-free site. There were significant differences among families in mortality rates in inoculated treatments but not in uninfected controls, as shown in the figure below. This demonstrates genetic variation in resistance to the fungus. Since the families showed a continuous range of mortality, quantitative genetic variation is present, rather than single-locus variation.



A seed orchard has been established to supply dieback-resistant jarrah for use in revegetation. Consequently, forest habitat will be maintained for threatened species. Further, other affected species (some of them rare or endangered) may also show genetic variation for resistance.

The four issues defined above are explored in detail in [Chapters 11–17](#). Here we introduce the concepts underlying quantitative genetics, its terminology, modes of analysis and prediction.

Molecular measures of genetic diversity do not answer many of the questions we wish to address in conservation genetics, as correlations between molecular and quantitative measures of genetic diversity are low (see below).

## Properties of quantitative characters

Quantitative characters typically show continuous rather than discrete distributions, are influenced by many loci and are strongly affected by the

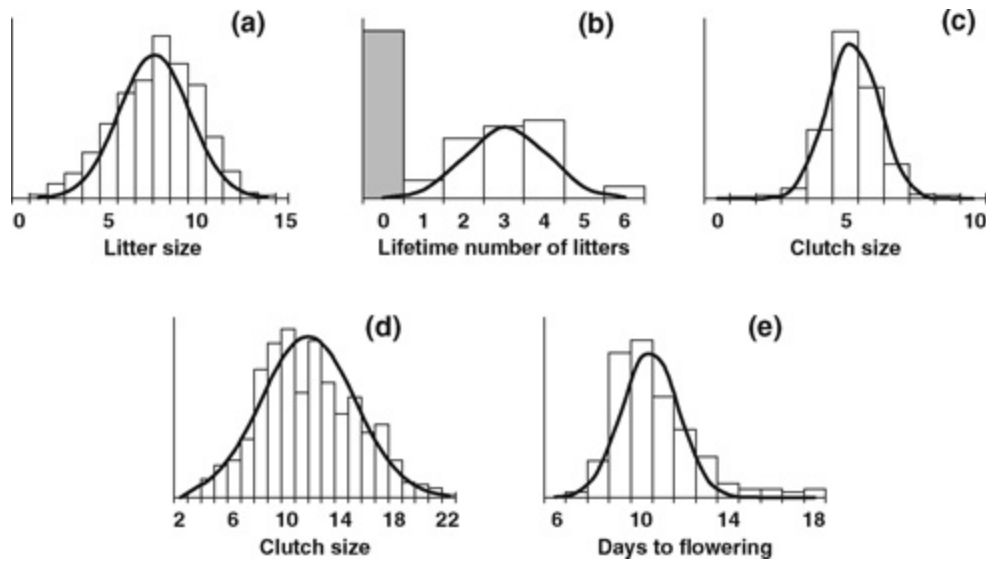


environment

Properties of quantitative characters are contrasted with those for qualitative, single-locus traits in [Table 5.1](#). Quantitative characters typically have continuous, approximately normal distributions, rather than discrete distributions ([Fig. 5.1](#)). Examples include characters such as reproductive fitness, longevity, height, weight, disease resistance, etc. They are influenced by many loci plus environmental influences, such as nutritional state (Lynch & Walsh [1998](#)). We are thus concerned with differences between individuals that are of degree, rather than of kind.

**Table 5.1** *Comparison of the characteristics of quantitative and qualitative characters*

Characteristic	Quantitative	Qualitative
Distributions	Unimodal and continuous	Multimodal and discrete
Genotype–phenotype relationship	Incomplete	Close
Loci	Many	One or few
Environmental effects	Often large	Usually small
Parameters for describing	Means, variances, $h^2$ , $V_A$	$p$ , $q$
Examples	Reproductive fitness, weight, height	Brown vs. yellow snail shells, Adh Fast vs. Slow electrophoretic mobility, DNA sequence differences



**Fig. 5.1** Distributions of phenotypes for five quantitative characters that are components of reproductive fitness: (a) litter size in mice, (b) lifetime production of litters per female in endangered black-footed ferrets, (c) clutch size in starlings, (d) clutch size in a rattlesnake and (e) days to flowering in *Arabidopsis* plants. Normal distributions are fitted to the data. The large number of non-breeding individuals in black-footed ferrets is probably due to poor adaptation to captive conditions. Data from (a) Falconer & Mackay (1996), (b) Russell (1999), (c) and (d) Wright (1968) and (e) Jones & Wilkins (1971).

The association between genotype and phenotype is typically weaker for quantitative than qualitative characters

It is not possible to directly infer genotype from observed phenotype for quantitative characters. Individuals with the same genotype may have different phenotypic values, and individuals with the same phenotypic value may have very different genotypes. For example, black-footed ferrets

producing six litters ([Fig. 5.1b](#)) will, on average, carry more alleles for large numbers of litters than those ferrets producing only one litter. However, both high and low groups will contain some animals whose reproductive performance is heavily influenced by positive or negative environmental influences during their development.

## Environmental variation

---

A proportion of the observed variation among individuals for quantitative characters is attributable to environmental, rather than genetic, causes

Environmental differences among individuals may arise from food supply, living conditions, disease status, etc. in animals, and differences in soil fertility, temperature, light, crowding, etc. in plants. For example, the endangered Seychelles warbler on Cousin Island averages 0.28 young per pair per year, but offspring production is 7.3 times higher in high-quality than in low-quality territories. When birds were translocated to Aride Island, where the insect food supply was over three times greater than Cousin Island, production for the same birds rose by a factor of 44 (Komdeur *et al.* [1998](#)).

## Basis of quantitative genetic variation

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Quantitative characters are typically influenced by genetic variation at many loci

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Genetic diversity for quantitative characters in outbred populations is due to the segregation of multiple, polymorphic Mendelian loci, referred to as **quantitative trait loci** (QTL) (Maloof 2006; Frankham 2009a). These loci possess alleles that add to, or detract from, the magnitude of the character. These loci individually show the usual Mendelian properties of segregation and linkage.

---

The heritability ( $h^2$ ) is used to measure genetic diversity for quantitative characters

---

The **heritability** of a character in a population is a major determinant of ability to evolve in response to selection. In its simplest form, this is the proportion of the total phenotypic variance in a population due to genetic differences among individuals. More specifically, heritability is the proportion of phenotypic variance attributable to genetic variation that parents can pass on to their offspring. Heritability is symbolized  $h^2$ , although the superscript 2 has no numerical meaning.

Two examples illustrate this concept. The Wollemi pine lacks genetic variation at hundreds of DNA marker loci, and all individuals tested were susceptible to dieback fungus (Peakall *et al.* 2003). Variation among trees is of entirely environmental origin ( $h^2 = 0$ ) and the capacity of this species to evolve is essentially zero. In contrast, resistance to root-rot fungus in jarrah trees has a significant heritability (Box 5.1), so jarrahs can evolve resistance.

## Methods for detecting quantitative genetic variation

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Genetic variation for quantitative characters can be detected using data from different genotypes compared under the same environmental conditions

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Three methods are used to determine whether a portion of phenotypic variance for quantitative characters derives from genetic variation among individuals:

- variation within and among populations
- comparisons of inbred with outbred populations
- resemblances among relatives.

Because of the major impact that environment can have on quantitative characters, it is critical that all comparisons be carried out contemporaneously in the same environment (a **‘common garden’ experiment**).

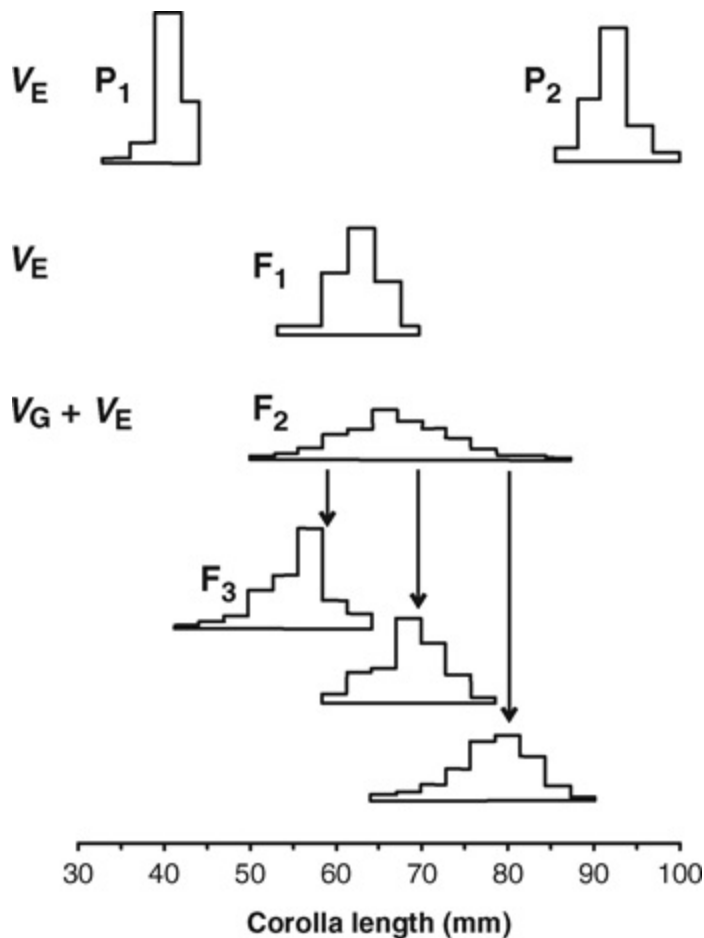
If phenotypic variation within inbred populations is less than that in outbred populations when both are raised under the same environment, then a genetic component underlying the trait is clearly present. This is illustrated for corolla length in tobacco ([Box 5.2](#)). In highly inbred populations, phenotypic variation is due solely to environmental causes, whilst variation in the  $F_2$  generation is due to both environmental and genetic heterogeneity among individuals. Quantitative genetic variation is the extent to which the outbred variation exceeds that within the genetically invariant genotypes (see Example 5.1).

---

**Box 5.2 Quantitative genetic variation for corolla length**

## in tobacco (after Strickberger 1985, based on East)

In a classical study on inheritance of a quantitative character, East crossed two highly inbred (homozygous) parental populations (P) of tobacco that differed in corolla length. He grew them and their  $F_1$  and  $F_2$  crosses contemporaneously in the same field.



Quantitative genetic variation in corolla length ( $V_G$ ) was shown by the difference in mean between the two parental populations and their non-overlapping distributions. Variances among individuals within the parental populations and within the  $F_1$  are environmental variance ( $V_E$ ).

The study demonstrated genetic variation for corolla length by a second means, as variation was greater in the  $F_2$  than in the parents or the  $F_1$ . For Mendelian loci affecting corolla length, we expect the  $F_2$  should show

greater variation than the parents and  $F_1$  due to allelic segregation, with all genotypes having similar environmental variation. For example, if the parental populations are  $A_1A_1$ , and  $A_2A_2$ , the  $F_1$  will all be  $A_1A_2$ , while the  $F_2$  will show segregation with  $\frac{1}{4}$   $A_1A_1$ ,  $\frac{1}{2}$   $A_1A_2$  and  $\frac{1}{4}$   $A_2A_2$ .

East verified that there were genetic differences among individuals in the  $F_2$  by breeding individuals with different corolla lengths and showing that their offspring differed on average in the same directions as the parents.

---

Phenotypic resemblances among relatives in genetically variable populations increase with the degree of similarity in their genetic makeup. Resemblances are greatest between identical twins (or clones), lower among full-sibs or between parents and offspring, and least among unrelated individuals. Perhaps the best known are analyses of personality traits, mental abilities, disease risk, etc. by comparisons of monozygotic (genetically identical) with dizygotic (average 50% genetic identity) twins in humans. If there is no heritable variation, then individuals with different degrees of relationship will show the same zero correlation. A significant resemblance (correlation or regression) among relatives demonstrates genetic variation for a quantitative character, as seen for shell width in endangered *Partula* snails (Box 5.3). Such resemblances among relatives have been demonstrated for innumerable characters in many outbreeding species of animals and plants.

## Partitioning genetic and environmental variation

---

Genetic and environmental variation can be partitioned using data from different genotypes or families compared under the same environmental conditions

---

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So far we have simply stated that the phenotypic value of an individual is the consequence of the alleles it inherits, together with the environmental influences it has encountered during its development. Algebraically, the above statement can be expressed:

$$P = G + E \quad (5.1)$$

where  $P$  = phenotype,  $G$  = genotype and  $E$  = environment. We now describe how the genetic component can be partitioned from the environmental component.

Phenotypic variance ( $V_P$ ) within a population represents the sum total of all contributions from genetic diversity ( $V_G$ ), the environment variation ( $V_E$ ), and interactions between genotypes and environment (often termed  $G \times E$  interactions):

$$V_P = V_G + V_E + 2Cov_{GE} \quad (5.2)$$

where  $Cov_{GE}$  is the covariance between genetic and environmental effects. This term is negligible when all individuals are raised under the same environmental conditions. A numerical example of partitioning variation is given in Example 5.1. Of the variation in corolla length in the  $F_2$ , 68% was due to segregation of polymorphic loci ( $V_G$ ) and 32% to environmental variation ( $V_E$ ). In a related manner, 85% of the variation in disease resistance in jarrah trees was heritable ([Box 5.1](#)).

---

### Example 5.1 Partitioning genetic and environmental



## variation

The variances for corolla length in tobacco for the data in [Box 5.2](#) are 48 and 32 for the two homozygous parent populations, 46 in the  $F_1$  population, and 130.5 in the  $F_2$ . The variances in both parents and the  $F_1$  are due solely to environmental variance,  $V_E$ . Consequently, we average the three separate estimates of  $V_E$ :

$$V_E = \frac{(V_{P_1} + V_{P_2} + V_{F_1})}{3} = \frac{(48 + 32 + 46)}{3} = 42$$

Variation in the  $F_2$  is due to both genetic diversity and environmental variation

$$V_{F_2} = V_G + V_E = 130.5$$

By rearranging this equation and substituting for  $V_E$ , we can estimate  $V_G$ , as follows:

$$V_G = V_{F_2} - V_E = 130.5 - 42 = 88.5$$

Thus,  $88.5/130.5 = 68\%$  of the total  $F_2$  variance in corolla length is due to segregation of polymorphic loci, and the remaining 32% is due to environmental variation.

---

Randomly allocating individuals across the range of environments to eliminate the covariance term in [Equation 5.2](#) is routinely carried out in quantitative genetic experiments with domestic animals and plants, where cultivation or rearing conditions can be standardized. However, this is difficult to achieve in wild populations. For example, in territorial species of birds and mammals, the genetically fittest parents may obtain the best territories (and the least fit, the poorest territories). Offspring inheriting the best fitness genotypes also ‘inherit’ the best environments, resulting in a positive genotype  $\times$  environment covariance and elevated phenotypic

resemblance among relatives.

Maternal environment influences a range of characters, especially in mammals. For example, mammal offspring from large litters typically weigh less than those from small litters due to less pre- and postnatal maternal provisioning per offspring. Embryo transfer, cross-fostering and standardization of litter sizes can be used to equalize maternal effects. Sire–offspring regressions are not affected by them, whilst dam–offspring regressions are. Mousseau & Fox (1998) provide further details on maternal effects.

## Partitioning of quantitative genetic variation

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Genetic diversity for quantitative characters is partitioned into components reflecting adaptive evolutionary potential ( $V_A$ ), dominance deviations ( $V_D$ ) and effects of outbreeding ( $V_I$ )

---

Genetic variance,  $V_G$ , is partitioned into **additive genetic variance** ( $V_A$ ), **dominance variance** ( $V_D$ ) and **interaction** variance ( $V_I$ ), as follows:

$$V_G = V_A + V_D + V_I \quad (5.3)$$

$V_A$  reflects variation in the average effects of loci,  $V_D$  measures variation in dominance deviations and  $V_I$  arises from variation in interaction (epistatic) deviations among gene loci. The first and third of these components have major conservation implications, as follows:

- $V_A$  and especially the ratio  $V_A/V_P$  (the heritability) reflect the adaptive evolutionary potential of the population for the character under study
- $V_I$  influences the effects of outbreeding, whether beneficial or deleterious.

In what follows, we indicate the genetic basis of these components of genetic variation and how they are measured (see also [Chapters 13](#) and [16](#)).

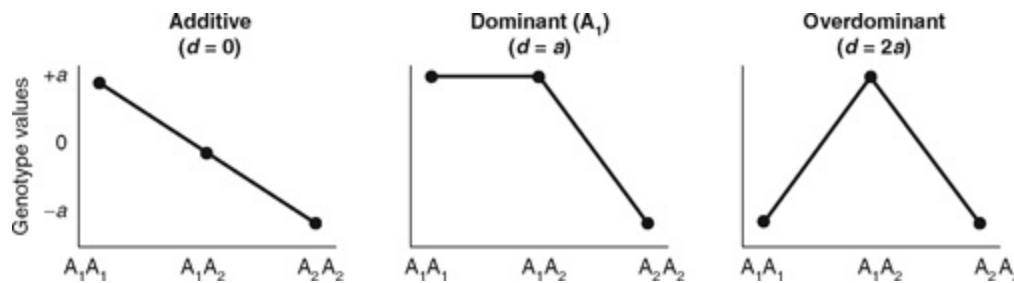
## A single-locus model illustrating partitioning of variance

To understand the meaning of  $V_A$  and  $V_D$ , we consider a single-locus model with two alleles. The three genotypes  $A_1A_1$ ,  $A_1A_2$  and  $A_2A_2$  are assigned genotypic values of  $a$ ,  $d$  and  $-a$  ([Fig. 5.2](#)). Homozygotes differ in genotypic value by  $2a$ , and the heterozygotes differ from the mean of the two homozygotes by  $d$ .

Genotype	$A_1A_1$	$A_1A_2$	$A_2A_2$
	----- ----- -----		
Genotypic values	$a$	$d$	$-a$
Frequencies	$p^2$	$2pq$	$q^2$

**Fig. 5.2** Genotypic values assigned to the three genotypes at a locus along with their frequencies with random mating.

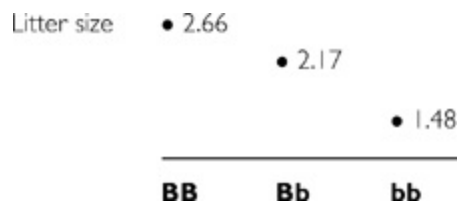
Genotypic values for **additive**, **dominant** and **overdominant** loci are illustrated in [Fig. 5.3](#). For an additive locus, heterozygotes are intermediate between homozygotes, so the three genotypes have values of  $a$ ,  $0$  and  $-a$ , with  $d$  being zero. If the  $A_1$  allele is dominant ( $A_2$  recessive), the genotypes have values  $a$ ,  $a$  and  $-a$ , respectively, so  $d = a$ . Conversely, if  $A_1$  is recessive, the genotypes have values  $a$ ,  $-a$  and  $-a$ , and  $d = -a$ . For the illustrated overdominant (heterozygote advantage) locus, the genotypic values are  $-a$ ,  $a$  and  $-a$ , so  $a = 0$  and  $d = 2a$ . Example 5.2 illustrates the computation of  $a$  and  $d$  for litter sizes at the Booroola locus (B) in Merino sheep. The B allele is partially dominant in this case.



**Fig. 5.3** Genotypic values for additive, dominant and overdominant loci.

### Example 5.2 Calculating $a$ and $d$ for mean litter sizes for genotypes at the Booroola locus in Merino sheep (after Lynch & Walsh 1998)

The Booroola (B) allele increases litter size in Merino sheep. Mean litter sizes for the three genotypes at the Booroola locus are:



The value of  $a$  is computed as:

$$a = \frac{\text{BB value} - \text{bb value}}{2} = \frac{2.66 - 1.48}{2} = 0.59$$

The mid-point of the genotypic value between the two homozygotes is the zero point on the scale in Fig. 5.2 and is required to determine  $d$ . It is  $(2.66 + 1.48)/2 = 2.07$ . Thus, the dominance deviation,  $d$ , is

$$d = \text{Bb value} - \text{mid-point} = 2.17 - 2.07 = 0.10$$

Thus, each copy of the Booroola allele increases litter size by an average of 0.59 lambs, and there is partial dominance, with the heterozygote having 0.10 lambs above the average for the two homozygotes.

---

## Additive genetic variance ( $V_A$ )

---

Additive genetic variation depends on the level of heterozygosity in the population and the average effects of alleles

---

For a polymorphic locus with additive effects ( $d = 0$ ) and frequencies and genotypic values as given in [Fig. 5.2](#), additive genetic variation is defined as:

$$V_A = 2pqa^2 \quad (5.4)$$

Thus, additive genetic variation depends on the heterozygosity ( $2pq$ ) in the population. Further,  $V_A$  depends on half the difference in mean between the homozygous genotypes ( $a$ ). The larger this difference between the two homozygotes, the larger the value of  $V_A$ .

When dominance exists ( $d \neq 0$ ), the additive genetic variation is:

$$V_A = 2pq [a + d (q - p)]^2 \quad (5.5)$$

Thus,  $V_A$  also depends on the dominance deviation,  $d$ .

$V_A$  for a character in a population is due to the combined impacts of all segregating loci with effects on the character.

## Evolutionary potential, additive variation and heritability

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The ability of a population to respond to selective forces (evolutionary potential) depends upon the heritability

Heritability determines the extent to which a character is passed on from parent to offspring. Traits that are beneficial in the parental generation will only get passed on to the offspring if they are heritable. For example, age at first reproduction in captive baboons is highly heritable; daughters of females who reproduce earlier also reproduce earlier (Williams-Blangero and Blangero 1995).

Heritability ( $h^2$ ) is defined as the proportion of total phenotypic variation due to additive genetic variation:

$$h^2 = \frac{V_A}{V_P} \quad (5.6)$$

Heritabilities range from 0 to 1. The former is found in highly inbred populations possessing no genetic variation, while the latter is expected for a character with no environmental variance in an outbred population, if all the genetic variation is additive.

A heritability estimate is specific to a particular character and population

in a particular environment

---

Heritabilities cannot be extrapolated to different environments as they are specific to a particular population living under specific environmental conditions. Populations with greater additive variation will have higher heritabilities when compared in the same environment. For example, populations of fruit flies lost allozyme genetic diversity over time, and their heritabilities for sternopleural bristle number decreased correspondingly (Briscoe *et al.* [1992](#)). Despite these provisos, heritability estimates show relatively consistent patterns in magnitude for similar characters among populations within species, and across species (see below).

## Estimating heritabilities

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Quantitative variation is characterized using statistical parameters including means, variances, covariances, etc.

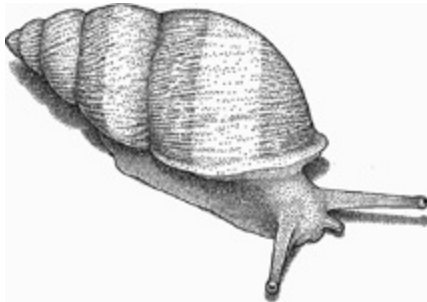
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To characterize quantitative variation, we partition the variation into genetic and environmental components, typically from studying the resemblances among relatives. Consequently, the parameters used to describe quantitative traits, and to partition its variation, are statistical values; means, variances, covariances, regressions and correlations in groups of organisms. These are described in [Box 5.3](#), using data from an endangered Tahitian land snail.

---

### Box 5.3 Statistics used to characterize variation for quantitative characters, illustrated using data for shell width in an endangered Tahitian snail

The following data describe the shell width for parents and offspring of 40 families of an endangered *Partula* snail originating from the island of Moorea in Tahiti (data from Murray & Clarke 1968). This and several other endangered species have been depleted due to predation by an introduced carnivorous snail. Each pair of data points represents the mean shell width of a female and male parent (P) together with the mean shell width (mm) in their offspring (O).



Partula snail from Tahiti

Parent means	Offspring means	Parent means	Offspring means	Parent means	Offspring means
P	O	P	O	P	O
6.8	7.3	7.5	7.3	7.8	7.5
6.9	7.4	7.6	7.7	7.9	7.6
6.9	7.6	7.6	7.7	7.9	7.7
7.1	7.5	7.6	7.9	7.9	7.7
7.3	7.3	7.6	7.4	7.9	7.7
7.3	7.2	7.6	7.5	7.9	7.8
7.3	7.4	7.6	7.4	8.0	7.7
7.4	7.7	7.7	7.6	8.0	7.9
7.5	7.6	7.7	7.9	8.0	7.8
7.5	7.5	7.8	7.5	8.1	7.8
7.5	7.7	7.8	7.8	8.1	7.8
7.5	7.4	7.8	7.9	8.1	7.9
7.5	7.8	7.8	7.6	8.5	8.1
7.5	7.6				

The **mean** shell length of parents  $\bar{P}$  is



$$\bar{P} = \frac{\sum_{i=1}^n P_i}{n} = \frac{(6.8 + 6.9 + \dots + 8.5)}{40} = 7.65 \text{ mm}$$

Similarly the mean for the offspring  $\bar{O}$  is

$$\bar{O} = \frac{\sum_{i=1}^n O_i}{n} = 7.63 \text{ mm}$$

The **variance** is a measure of the spread of the data around the mean. The phenotypic variance for the parents,  $V_P$ , is

$$V_P = \frac{\sum_{i=1}^n (P_i - \bar{P})^2}{(n - 1)} = \frac{[(6.8 - 7.65)^2 + \dots + (8.5 - 7.65)^2]}{(40 - 1)} = 0.125$$

The variance for the offspring  $V_O$  is

$$V_O = \frac{\sum_{i=1}^n (O_i - \bar{O})^2}{(n - 1)} = \frac{[(7.3 - 7.63)^2 + \dots + (8.1 - 7.63)^2]}{(40 - 1)} = 0.043$$

The **standard deviation** is a measure of spread around the mean and is the square root of the variance. Standard deviations for parents and offspring,  $SD_P$  and  $SD_O$ , are computed as follows:

$$SD_P = \sqrt{0.125} = 0.354$$

$$SD_O = \sqrt{0.043} = 0.207$$

Often a data set is described as mean  $\pm$  SD, so for parents shell width is  $7.65 \pm 0.35$ .

The **covariance** between offspring and parents  $Cov_{PO}$  measures the extent to which they vary in concert (+), vary independently (0), or in opposition (-). It is defined as

$$\begin{aligned} Cov_{PO} &= \frac{\sum_{i=1}^n (P_i - \bar{P})(O_i - \bar{O})}{(n - 1)} \\ &= \frac{[(6.8 - 7.65)(7.3 - 7.63) + \dots + (8.5 - 7.65)(8.1 - 7.63)]}{(40 - 1)} \\ &= 0.050 \end{aligned}$$

The **correlation**  $r_{PO}$  between offspring and parents is a standardized measure of the extent to which they vary in concert. Correlations range from  $-1$  to  $+1$ . It is defined as

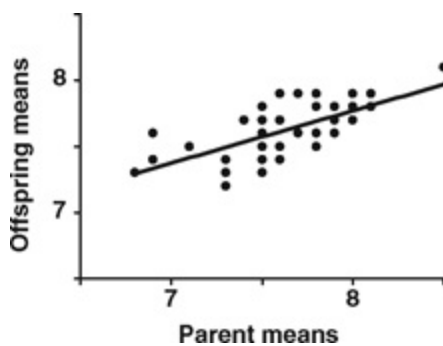
$$r_{PO} = \frac{Cov_{PO}}{\sqrt{(V_P \cdot V_O)}} = \frac{0.050}{\sqrt{(0.125 \times 0.043)}} = 0.679$$

Thus, there is a positive correlation between offspring and parents; parents with wider shells have offspring with wider than average shells, while parents with narrower shells have offspring with narrower than average shells.

The **regression** of offspring on parent ( $b_{OP}$ ) is the slope of the line of best fit relating offspring and parents, as shown in the figure above. It is defined as

$$b_{OP} = \frac{Cov_{PO}}{V_P} = \frac{0.050}{0.125} = 0.40$$

This regression is of major importance as it measures the degree to which variation is due to additive genetic causes (heritability). The heritability is 40% for *Partula* shell width, meaning that 40% of the observed variation is due to additively inherited differences and 60% to minor differences in the environment experienced by different individuals. Heritability is discussed in detail below.



Further details about the above measures are given in statistics textbooks. They are easily calculated using statistical or spreadsheet software (see end of chapter) or scientific calculators.

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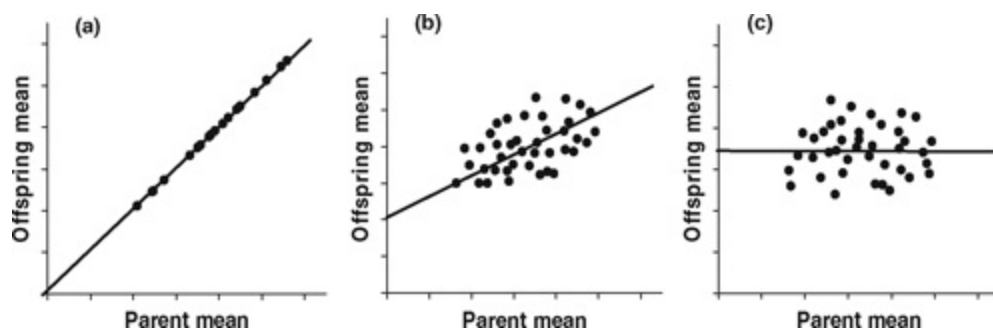
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The slope of the relationship between offspring means and parent means is a direct measure of the heritability ( $h^2$ ) of a trait

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Heritability and  $V_A$  are fundamental measures of how well quantitative traits are transmitted from one generation to the next. Figure 5.4 illustrates three contrasting strengths of relationship between parents and offspring and the corresponding differences in heritabilities. Figure 5.4a shows an example of complete inheritance. Parents with larger than average values for the trait produce offspring with larger values, while smaller than average parents produce smaller than average offspring. This trait has a high heritability = 1. In this case, the slope defining the relationship of parent mean to offspring mean (the regression coefficient) is 1. In this example, environmental differences among parents, and among offspring, have negligible influence on the phenotype for the trait. Fingerprint ridge count in humans approaches this level of relationship.



**Fig. 5.4** Hypothetical relationships between mean values of parents and mean values of offspring for three cases, representing (a) complete, (b) incomplete and (c) zero relationships between parents and offspring. The solid lines are best-fitting lines relating parents and offspring.

Inheritance is less complete in Fig. 5.4b. Parents with high phenotypic values produce offspring with higher than average values, but closer to the population mean than the parents themselves. Parents with low values produce offspring with higher values than themselves, but lower than the average. The slope of the relationship between offspring mean and parent mean is  $<1$  and the heritability has an intermediate value. Some of the superiority or inferiority of the parents is due to environmental, rather than genetic, effects and therefore cannot be inherited by their offspring. Many quantitative characters have relationships of this kind, including shell width in *Partula* snails (Box 5.3) and body size in many species, including endangered cotton-top tamarins (Cheverud *et al.* 1994).

There is no relationship between parent and offspring values in Fig. 5.4c. The slope of the relationship is 0 and  $h^2 = 0$ . Parents with high and low values of the trait have similar offspring with values randomly distributed around the mean. In this case  $h^2$  is zero. Such relationships are found in homozygous populations, such as the Wollemi pine, where all differences among parents are of environmental origin. Further, some reproductive characters in outbred populations, such as conception rate in cattle, approach this value.

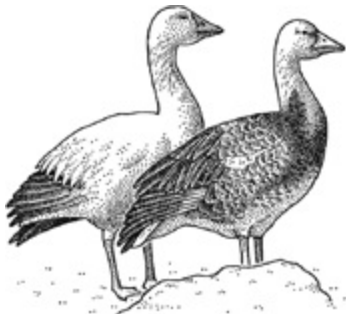
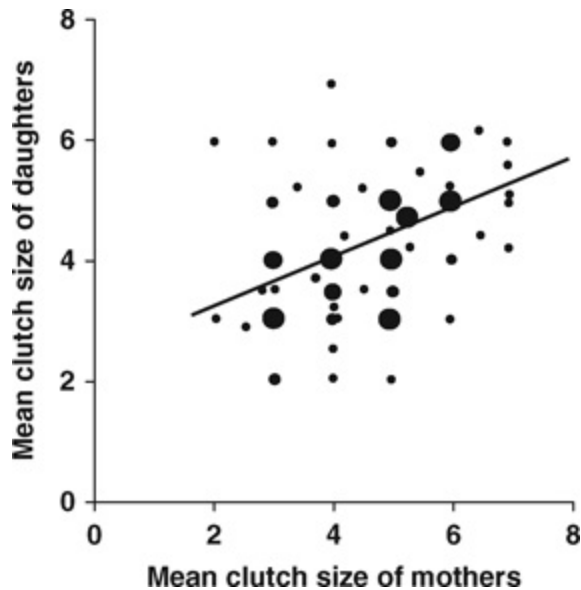
Heritabilities can also be estimated using regression of offspring mean on that of one parent, from full-sib correlations, and from half-sib correlations. In each case, the degree of genetic relationship between the relatives must be taken into account. For example, the heritability of a trait equals the regression of offspring on mid-parent, twice the regression of offspring on one parent (Example 5.3), twice the full-sib correlation and four times the half-sib correlation. Falconer & Mackay (1996) and Lynch & Walsh (1998) provide further details of these methods.

---

**Example 5.3 Estimating the heritability for clutch size in lesser snow geese from regression of daughter's mean on**

## mother's mean

In the figure below mean clutch sizes of daughters are plotted against those of their mothers for 132 mother–daughter pairs of lesser snow geese from Canada (Findlay & Cooke [1983](#)).



Lesser snow geese

The regression of daughter's mean clutch size ( $D$ ) on mother's mean clutch size ( $M$ ) is

$$D = 2.538 + 0.306 M$$

The slope of this line, shown on the figure, is equal to half the heritability, so we estimate heritability as:

$$h^2 = 2 \times 0.306 = 0.612$$

Thus, the heritability of clutch size in the lesser snow goose is 61%, indicating that mean clutch size can rapidly adapt towards its optimum value in this environment.

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## Predicting response to selection

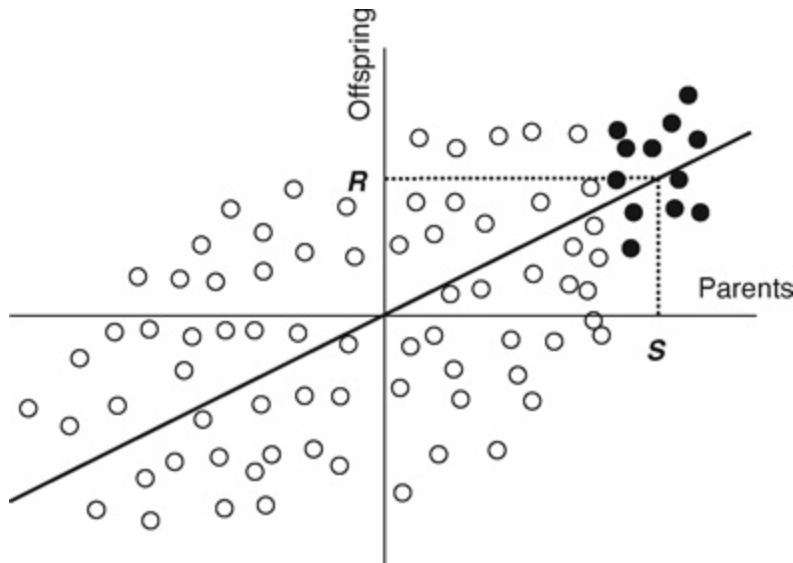
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Response to selection for a quantitative character depends on the intensity of selection applied and the heritability of the character

The genetic change (response) produced by **directional selection** (selection favouring one extreme) can be predicted from the intensity of selection, and the heritability, as illustrated graphically in [Fig. 5.5](#). Individuals with the highest value are selected to be parents and these parents have a mean  $S$  units above the mean of all individuals in the parent generation.  $S$  is termed the **selection differential**. Some of this superiority is due to environmental effects and some to genotypic differences, the heritability being the proportion due to additive genetic effects. Consequently, response to selection  $R$  (the difference in the offspring of selected parents compared to that of offspring of all parents) is

$$R = Sh^2 \quad (5.7)$$

Thus, selection response (evolutionary change) will occur if selection is applied ( $S > 0$ , or  $S < 0$ ), and there is additive genetic variation for the character ( $h^2 > 0$ ). There is no selection response in highly inbred (homozygous) populations lacking genetic variation ( $h^2 = 0$ ).



**Fig. 5.5** Predicting response to directional selection. The plot of values for a quantitative character in offspring is given against the mean of their parents (filled circles) for a quantitative character. The angled line is the linear regression of offspring mean on mid-parent mean (slope of  $h^2$ ). Using basic geometry, we can predict response to selection by raising a perpendicular from the mean of selected parents  $S$  on the  $x$ -axis to the regression lines, and then dropping a perpendicular from this point to  $y$ -axis (at  $R$ ).  $R$  is the superiority of the offspring of selected parents compared to those from all parents. *Genetic change is predicted to be  $S \times \text{slope of the line}$  ( $h^2$ ).*

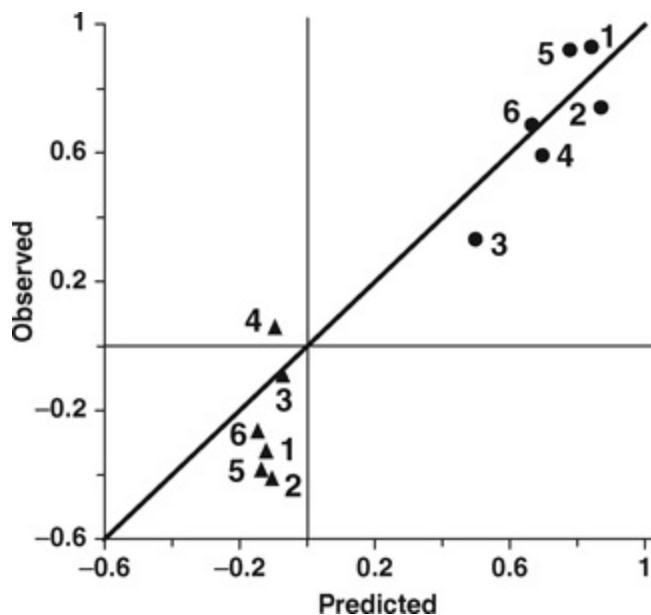
Example 5.4 illustrates prediction of response to selection for Darwin's medium ground finch from the Galápagos Islands. The predicted change in bill width (response to selection) is positive when parents have wider than average bill sizes and negative when they are narrower.

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The agreement between predicted and observed selection response for quantitative characters is usually good

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In general, [Equation 5.7](#) provides reasonable predictions of short-term selection response, especially for characters peripheral to fitness (**peripheral characters**) (Falconer & Mackay [1996](#); Roff [1997](#)). For example, [Fig. 5.6](#) shows good agreement between observed and predicted selection response for Darwin's medium ground finch in the Galápagos where the forces of natural selection vary across years, largely as a result of El Niño climatic cycles.



**Fig. 5.6** Comparison of observed and predicted response to natural selection in Darwin's medium ground finch on the Galápagos Islands (after Roff [1997](#), based on Grant & Grant). The straight line represents perfect agreement between observed and predicted selection response. 1, weight; 2, wing length; 3, tarsus length; 4, bill length; 5, bill depth; and 6, bill width.

Predictions from [Equation 5.7](#) are strictly valid for only one generation, as the genetic makeup of the population will change as a consequence of selection. In practice, it provides reasonable predictions for at least five generations (Falconer & Mackay [1996](#)). Importantly, the prediction applies only to the particular population under the environmental conditions where the heritability was measured.



### Example 5.4 Predicting response to directional selection for bill width in Darwin's medium ground finch on the Galápagos Islands (after Grant & Grant [1995](#), [2000](#))

Over an 18-month period of drought and no breeding from mid 1976 to end 1978, the finches suffered 85% mortality. Survivors had beaks that were 0.25 mm wider than the original population ( $S$ ). The heritability for bill width in this population is 74.5%. Hence, we predict a genetic change in the offspring of these survivors ( $R$ ), as follows:

$$R = S h^2 = 0.25 \times 0.745 = 0.19 \text{ mm}$$

In reality, an increase of 0.25 mm was observed in the average bill width of the offspring, compared to the expected 0.19 mm.



Darwin's medium ground finches from Galapagos

While the individual loci responsible for these genetic changes have not been identified, two loci with large effects on beaks (bone morphogenetic protein 4 and calmodulin) have been found. They differ in expression between species of Darwin's finches with large differences in beak size and shape (Abzhanov *et al.* [2006](#)).

Responses to selection for fitness characters are usually asymmetrical with poorer response for increased than reduced fitness

While [Equation 5.7](#) predicts symmetrical response to selection in the high and low directions, reproductive fitness characters typically show less response to selection for increased than for reduced fitness (Frankham [1990](#)). Asymmetrical response to selection for fitness traits is not surprising, as reproductive fitness is subject to directional natural selection. Thus, we would expect less response for improved than reduced fitness.

## Magnitudes of heritabilities

Most quantitative characters in naturally outbreeding species show heritable variation ( $h^2 > 0$ )

Most estimates of heritabilities for a range of fitness, size and beak characters in natural populations of birds have  $h^2 > 0$  ([Table 5.2](#)). A similar conclusion applies for essentially all outbred populations of animals and plants. For example, heritabilities for 98 quantitative traits in humans are all greater than zero (Pilia *et al.* [2006](#)).

**Table 5.2** *Heritabilities of fitness, body size and beak size for wild birds. Values should range from 0% to 100%, but values greater than 100%, or less than 0% can arise due to sampling variation in small experiments. Parent–offspring environmental correlations and biases due to maternal effects can also lead to values of greater than 100%*

Species	$h^2$ (%)		
	Fitness	Body size	Bill size
Canada goose		11	46
Collared flycatcher	−5, 0, 29, 32	47, 59	35, 48, 56, 40, 44
Darwin's medium ground finch	−17	56	84
Darwin's cactus finch		37, 110, 126	2, 13, 44, 129
Darwin's large cactus finch		54, 95	67, 69, 104, 137
European starling	34	49 <sup>a</sup>	
Great tit	37, 48	59, 59, 61, 64, 76	49, 71, 68
Penguin		92	76
Pigeon		28, 50	50, 58
Red grouse	30	35, 50	
Song sparrow		27, 36, <sup>a</sup> 71, 101 <sup>a</sup>	40, <sup>a</sup> 123, 71, 59
<b>Means</b>	<b>21</b>	<b>61</b>	<b>67</b>

<sup>a</sup> Progeny cross-fostered to minimize postnatal maternal effects.

*Sources:* After Smith (1993); Weigensberg & Roff (1996); Lynch & Walsh (1998); Keller *et al.* (2001).

Heritabilities are consistently lower for characters related to reproductive fitness than for other characters

Averages of heritabilities for wild bird species were 21% for fitness characters, 61% for size and 67% for peripheral (beak size) characters (Table 5.2). The average of estimates from humans, domestic and laboratory species also indicate lower heritabilities for fitness characters than for size or peripheral characters (Table 5.3), as do studies of wild populations of mammals, birds and fish (McCleery *et al.* 2004; Funk *et al.* 2005). The lower heritabilities for fitness than peripheral characters are due to higher environmental and non-additive variation, rather than lower additive genetic

variation.

**Table 5.3** *Mean heritabilities for different characters in (1) humans, domestic and laboratory animals, (2) animals excluding fruit flies, (3) fruit flies and (4) plants, based on reviews and meta-analyses*

Species group	$h^2$ (%)			
	Fitness	Size	Peripheral/Sundry	
(1) Mean of humans, domestic and laboratory animals	11	50	48	
	Life history	Behaviour	Physiology	Morphology
(2) Animals (excluding fruit flies)	26	30	33	46
(3) Fruit flies	12	18	–	32
(4) Plants			43	23 <sup>a</sup> , 39 <sup>b</sup>

**a** Vegetative morphology.

**b** Floral morphology.

*Sources:* (1) after Strickberger (1985); Falconer & Mackay (1996). (2) and (3) Roff (1997). (4) Ashman & Majetic (2006).

Heritabilities are typically lower in selfing species than in outcrossing ones

As selfing species have lower levels of molecular genetic diversity, it is not surprising that quantitative genetic variation in highly selfing species is only about 1/3–1/4 of that in outbreeding species (Hamrick & Godt 1989; Charlesworth & Charlesworth 1995). We shall return to the reasons for these

in [Chapters 11](#) and [12](#).

---

Estimates of heritabilities are imprecise unless they are based on hundreds to thousands of individuals

---

Individual heritability estimates for diverse characters in different species vary widely ([Table 5.2](#)). Much of this variation is due to the large standard errors on individual estimates. The precision of heritability estimates depends on the number of families, the total number of individuals studied and on the magnitude of the heritability itself. In general, several hundred to a few thousand progeny from 30 or more parental groups need to be measured to obtain precise estimates of heritabilities (Falconer & Mackay [1996](#)).

## **Heritabilities in endangered species**

Very few heritability estimates have been made for endangered species ([Table 5.4](#)). Since molecular measures of genetic diversity are generally lower in endangered than in related, non-endangered species ([Chapter 3](#)), we might expect heritabilities to differ in the same direction. In fact, four of the five heritability estimates are lower in endangered species than for comparable characters in non-endangered species, but there are insufficient data to resolve the issue. There is clearly a need for many more estimates of heritabilities to be made in threatened species.

**Table 5.4** *Heritabilities in endangered and in comparable non-endangered species*

Endangered species	Character	$h^2(\%)$	Non-endangered species	Character	$h^2(\%)$
Cotton-top tamarin	Body weight	35	Laboratory and domestic animals	Body size	50
Snails					
<i>Partula taeniata</i>	Shell length	36	<i>Arianta arbustorum</i>	Shell width	70
	Shell width	40			
<i>Partula suturalis</i>	Shell length	81			
	Shell width	53			

Sources: Murray & Clarke (1968); Cook (1965); Cheverud *et al.* (1994).

## Dominance variance ( $V_D$ )

Dominance variance depends on the heterozygosity and the dominance deviation  $d$

For a locus with the effects and frequencies defined in Fig. 5.2, dominance variance is (Falconer & Mackay 1996):

$$V_D = (2pqd)^2 \quad (5.8)$$

Dominance variance is present at a segregating locus if alleles show some degree of dominance ( $d \neq 0$ ). The response of a character to inbreeding depends on  $2pqd$  and the extent of inbreeding (Chapter 12) and so is related to  $V_D$ .

$V_D$  in a population is due to the combined impacts of all segregating loci exhibiting dominance effects on the character.

## Estimating $V_D$

$V_D$  is typically estimated from the differences between full-sib and half-sib covariances

To estimate  $V_D$ , data need to be collected on many groups of full-sibs and many groups of paternal half-sibs. As the covariance between full-sibs depends both on both  $V_A$  and  $V_D$ , while the covariance between half-sibs is dependent only on  $V_A$ , we can obtain an estimate of  $V_D$  by subtracting 8 times the half-sib covariance from 4 times the full-sib covariance (Table 5.5). This estimate is dependent upon there being no common environmental effects ( $V_{Ec}$ ) contributing to similarities among full-sibs.

**Table 5.5** *Covariances between full- and half-sibs and their composition in terms of  $V_A$ ,  $V_D$  and  $V_{Ec}$ . Estimation of  $V_D$  is shown in the bottom portion of the table*

Relatives	Covariance
Full-sibs	$Cov_{FS} = \frac{1}{2} V_A + \frac{1}{4} V_D + V_{Ec}$
Half-sibs	$Cov_{HS} = \frac{1}{4} V_A$
	$4 Cov_{FS} = 2 V_A + V_D + 4 V_{Ec}$
	$-8 Cov_{HS} = 2 V_A$
	$= V_D + 4 V_{Ec}$

Source: After Falconer & Mackay (1996).

## Magnitude of $V_D$

Reproductive fitness characters typically show greater dominance variance than other characters

Non-additive genetic variation is highest for fitness characters and lowest for peripheral characters (Table 5.6).

**Table 5.6 Dominance variance for life history (fitness), behavioural, physiological and morphological characters, expressed as a percentage of genetic variance ( $V_D/V_G$ ) and as a percentage of phenotypic variance ( $V_D/V_P$ ), based upon a meta-analysis. *Values for life history characters are higher on average than for other characters***

Measure	Life history	Behaviour	Physiology	Morphology
$V_D/V_G$	54	24	27	17
$V_D/V_P$	31	4	21	13

Source: Crnokrak & Roff (1995).

## Interaction variance ( $V_I$ )

If loci in a multilocus genotype are acting independently then the overall effect is simply the sum of the average effects of the genotype at each locus. However, epistatic interactions among loci can generate deviations evident as interaction variance ( $V_I$ ).  $V_I$  is one of the components determining whether crossing of populations has deleterious or beneficial effects (Chapter 16).

In practice, the partitioning of genetic variance is generally into additive ( $V_A$ ) and non-additive ( $V_D + V_I$ ) components, as it is very difficult to separate  $V_D$  and  $V_I$ . Readers are referred to Falconer & Mackay (1996) and Lynch &



Walsh (1998) for further details.

## Measuring genetic changes over time

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Detection of genetic changes over time requires that comparisons be made with a genetically stable control population, or by using contemporary comparisons using stored seed or cryopreserved embryos

---

To detect genetic changes over time (e.g. inbreeding depression, or change due to selection) randomly mated outbred populations, related species or samples derived from stored seed or cryopreserved embryos must be maintained as controls to detect environmental fluctuations from generation to generation.



Bighorn sheep

An elegant example of measuring changes over time is provided by Coltman *et al.* (2003) who showed that trophy hunting of bighorn sheep with large horns in Canada resulted in genetic reductions in body and horn size.

## Correlations between molecular and quantitative genetic

## variation

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Correlations between quantitative genetic variation and molecular measures of genetic diversity are low and are zero for life history traits

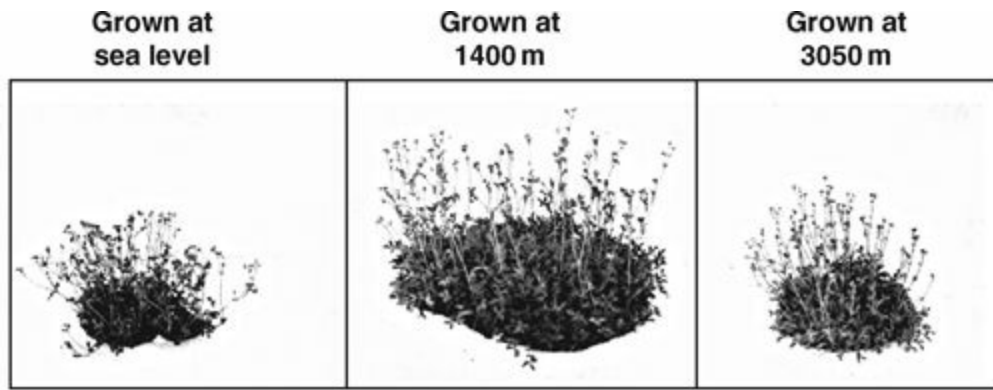
Since additive genetic variation is a function of heterozygosity, it might be expected that molecular estimates of heterozygosity and quantitative genetic variation would be closely correlated. However, the average correlation between them was only 0.21, based on a meta-analysis (Reed & Frankham [2001](#)). Further, the correlation did not differ significantly from zero for life history traits (−0.09). As selection operates on reproductive fitness (life history) characters but has little impact on molecular genetic markers, it is probably an important reason for the low correlation. Further, the low precision of estimates of genetic variation, especially for quantitative characters, also contributes to the weak relationship (Gilligan *et al.* [2005](#)). Consequently, molecular measures of genetic variation provide, at best, only a very imprecise indication of quantitative genetic variation and evolutionary potential.

## Genotype × environment interaction

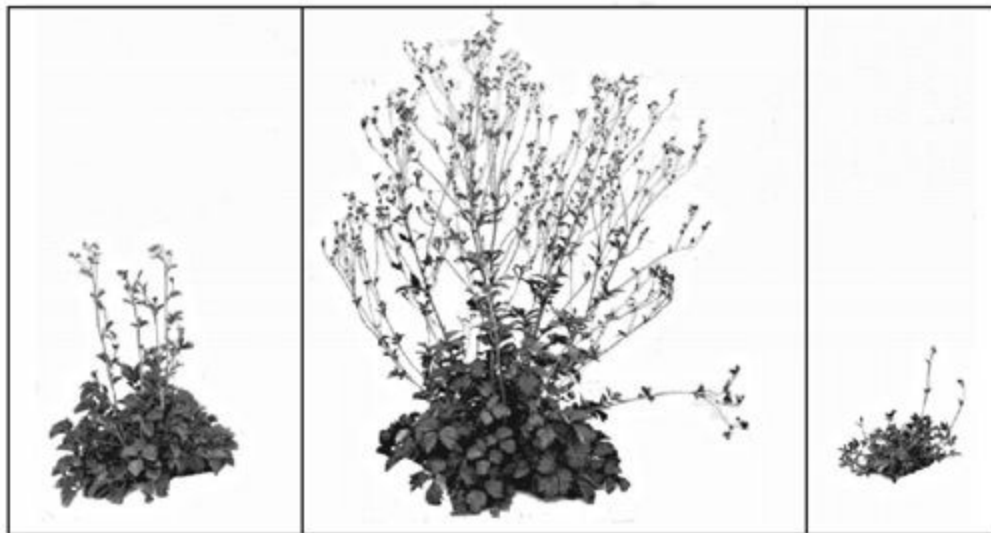
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Genotypes often show different relative performances in different environments

Differences in relative performance of genotypes in different environments are referred to as **genotype × environment interactions**. They typically arise when populations adapt to particular environmental conditions, and survive and reproduce better in their native conditions than in other environments. This is illustrated by the growth and survival of transplanted individuals of the sticky cinquefoil plant from high, medium and low elevations in California ([Fig. 5.7](#)).



*Potentilla g. nevadensis* from 3050 m



*Potentilla g. hanseni* from 1400 m



*Potentilla g. typica* from sea level

**Fig. 5.7** Genotype x environment interaction in the sticky cinquefoil plant. Strains of cinquefoil derived from high, medium and low altitudes were transplanted into their native and different locations in California and their growth and survival monitored (Clausen *et al.* 1948). *Populations generally*

*grow best in their own environment and poorest in the environment most dissimilar from their own.*

Genotype  $\times$  environment interactions are of major significance to the genetic management of endangered species, because:

- the reproductive fitness of translocated individuals cannot be predicted if there are significant genotype  $\times$  environment interactions
- success of reintroduced populations may be compromised by genetic adaptation to captivity – superior genotypes under captive conditions typically perform poorly when released into the wild
- mixing of genetic material from fragmented populations adapted to different environments may generate genotypes that do not perform well under some, or all, conditions
- knowledge of genotype  $\times$  environment interaction can strongly influence the choice of populations for return to the wild.

These issues are discussed in [Chapters 16, 17, 19 and 20](#).

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Genotype  $\times$  environment interactions are most common when genotypic differences and environmental differences are large

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Genotype  $\times$  environment interactions are most likely to be detected in species with wide geographic, ecological or altitudinal ranges (Frankham & Weber 2000) ([Fig. 5.7](#)). Further, quantitative traits closely associated with reproductive fitness appear to be more prone to genotype  $\times$  environment interactions than peripheral characters. Genotype  $\times$  environment interactions are more likely in plants than animals, as plants are less mobile and often

exhibit strong local genetic adaptations to their immediate environment (soil chemical composition, grazing pressure, wind, etc.) (Briggs & Walters 1997).

## Summary

1. Characters of importance in conservation biology are primarily quantitative, especially reproductive fitness.
2. Variation for quantitative characters among individuals is due to both genetic and environmental effects.
3. Groups of individuals must be studied for quantitative characters, and statistical analyses used to study and partition variation.
4. Genetic diversity for quantitative characters is due to the segregation of multiple quantitative trait loci (QTL).
5. The evolutionary potential of a character in a population is determined by its additive genetic variation and heritability (the additive genetic variation as a proportion of the total variation).
6. Heritabilities are typically lower for reproductive fitness characters than for characters peripheral to fitness.
7. Heritabilities are typically estimated from the resemblances between relatives for quantitative characters e.g. offspring–parent regressions and half-sib correlations.
8. Variance due to dominance deviations is greater for fitness than for peripheral characters.
9. The interaction variance is important in the context of crossing different populations.
10. Different genotypes may show altered ranking in different environments (genotype  $\times$  environment interactions); this is important in the context of translocating individuals.

## Further reading

Falconer & Mackay (1996) *Introduction to Quantitative Genetics*. An excellent readable textbook on quantitative genetics.

Frankham (1999) Review on quantitative genetics in conservation biology.

Frankham (2009a) Review on the genetic architecture of quantitative genetic

variation.

Lynch & Walsh (1998) *Genetics and Analysis of Quantitative Traits*. A comprehensive advanced treatment of quantitative genetics.

Roff (1997) *Evolutionary Quantitative Genetics*. A readable textbook on quantitative genetics with an evolutionary focus.

## Software

Regressions, correlations and analyses of variance can be computed using a range of general software packages, including:

GENSTAT: Commercial software for a broad range of statistical analyses.  
[www.vsni.co.uk/products/genstat/](http://www.vsni.co.uk/products/genstat/)

MINITAB: Commercial software for a broad range of statistical analyses.  
[www.minitab.com/](http://www.minitab.com/)

SAS/STAT: Commercial software for a broad range of statistical analyses.  
[www.sas.com/technologies/analytics/statistics/stat/index.html](http://www.sas.com/technologies/analytics/statistics/stat/index.html)

VASSARSTATS: Free software for statistical analyses, including analyses of variance, correlations and regressions.  
<http://faculty.vassar.edu/lowry/VassarStats.html>

## Problems

- 5.1 Statistics. Compute the parent and offspring means and variances and the covariance between parent and offspring values for the following data set on shell size in 21 families of endangered *Partula* snails from Tahiti (data from Murray & Clarke 1968). Each pair of data represent the mean shell length of the parents (P) and their offspring (O) for a single family.

P	O	P	O
18.5	17.5	20.1	18.5
18.6	18.3	20.3	21.6
18.7	19.1	20.3	18.9
18.9	19.0	20.4	19.3
19.2	18.7	20.7	21.3
19.4	17.4	20.7	20.7
19.6	18.3	20.7	19.1
19.8	19.2	21.2	20.3
19.8	17.8	21.2	19.3
19.9	19.4	21.4	20.2
20.0	19.9		

- 5.2 Heritability. Compute the regression of offspring on parent for the data on shell length in *Partula* snails in Problem 5.1. Compute the heritability from the regression coefficient. Plot the relationship and insert the regression line.
- 5.3 Heritability. For the following hypothetical data on lifetime offspring numbers in seven families of endangered California condors, compute the regression of offspring mean on parent mean and calculate the heritability. Plot the relationship between offspring mean and parent mean and insert the regression line.

Family	1	2	3	4	5	6	7
Offspring mean	5.4	5.6	5.8	6.0	6.2	6.4	6.6
Parent mean	3.0	4.0	5.0	6.0	7.0	8.0	9.0

- 5.4 Heritability. If the slope of the regression of offspring body size on father's body size in the Barnacle goose is 0.27, what is the heritability of body size in this population (Weigensberg & Roff 1996)?
- 5.5 Genotypic values. Specify the genotypic values for a locus with alleles  $A_1$  and  $A_2$  where  $A_2$  is recessive (in terms of the symbols  $a$ ,  $d$  and  $-a$ ), similar to Fig. 5.3.
- 5.6 Relationship between  $V_A$  and heterozygosity. Determine the expression for  $V_A$  for a single additive locus below

Genotype	$A_1A_1$	$A_1A_2$	$A_2A_2$
Genotypic values	$a$	$0$	$-a$
Frequencies	$p^2$	$2pq$	$q^2$

First compute the mean and then compute the genotypic variance



$$V_A = \frac{\sum f_i (X_i - \bar{X})^2}{\sum f_i}$$

- 5.7 Heterozygosity and quantitative genetic variation. What happens to  $V_A$ ,  $V_D$  and  $h^2$  if the heterozygosity increases by 10%?
- 5.8 Heterozygosity and quantitative genetic variation. What happens to  $h^2$ ,  $V_A$  and  $V_D$  if heterozygosity drops by 50%?
- 5.9 Selection differential. If the breeding individuals for an endangered species in captivity have a mean 'wildness' score of 8, and the whole population has a mean of 10, what is the selection differential (S)?
- 5.10 Response to selection. What is the expected response to selection for body size in cotton-top tamarins if individuals producing offspring have a mean body size of 410 g, while the population mean body size is 450 g and the heritability of body size is 35%?
- 5.11 Response to selection. What is the expected response to selection for depth of bill in Darwin's medium ground finch on the Galápagos Islands due to the drought in 1977 (Grant & Grant [1995](#), [2000](#))? The heritability of bill depth in this population is 0.73. Bill depth was 9.42 mm before the drought and 9.96 mm in those that survived the drought.

## Chapter 6 Evolutionary impacts of natural selection in large populations

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Species must evolve to cope with environmental change. Adaptive evolutionary changes in large natural populations occur through selection increasing the frequency of beneficial alleles

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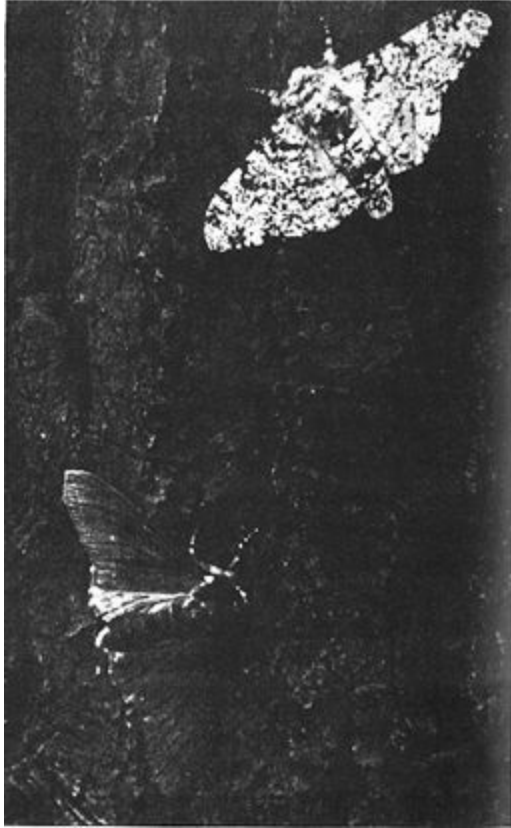
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### Terms

Adaptive evolution, convergent evolution, directional selection, disruptive selection, ecotype, fitness, fixation, lethal, natural selection, partial dominance, phenotypic plasticity, relative fitness, selection coefficient, stabilizing selection

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Industrial melanism in the peppered moth; peppered and melanic (black) moths on trees in polluted and unpolluted areas (Europe). The melanic form is better camouflaged in the polluted area and the peppered moth in the unpolluted area

(from Kettlewell [1973](#))

## The need to evolve

Environmental change is a ubiquitous feature of the conditions

experienced by species. Consequently, they need to evolve to avoid extinction

---

Species have to cope with continual changes over time in both the physical and biotic environments (pests, parasites, diseases and competitors), and especially changes wrought by human activities. Global climate change is now occurring as a consequence of the burning of fossil fuels (UNEP 2007). Impacts of global warming on living organisms are already evident in data from several hundred species including coral reef bleaching (Parmesan & Yohe 2003; Root *et al.* 2003). On the geological timescale, there are major climatic shifts between ice ages and warm periods.

Disease organisms evolve new strains and spread to new locations, new diseases arise and pathogens switch hosts (Garrett 1994). Adaptations in competitors, pests and parasites mean that species must continually evolve to avoid falling behind competing organisms (the 'Red Queen' hypothesis: Van Valen 1973).

Alterations in disease organisms have been the most pervasive and frequent environmental change faced by most species. For example, 300 major disease outbreaks affecting humans are recorded in Chinese history between 243 BC and 1911, approximately one every seven years (McNeill 1976). Plague killed nearly 20 million Europeans in the fourteenth century, while rinderpest eliminated 95% of the great wildebeest and Cape buffalo herds in East Africa in the 1890s (O'Brien & Evermann 1988; K. F. Smith *et al.* 2006). New influenza strains arise every few years and spread throughout the world, the H5N1 strain of bird flu being a recent example. Many diseases have crossed species boundaries, including HIV-1 (a cause of AIDS) from chimpanzees to humans, canine distemper from dogs to lions in Africa and to black-footed ferrets in the USA, and Hendra virus from fruit bats to horses and humans in Australia (Daszak *et al.* 2000).

Human activities are also spreading disease organisms from their original locations. For example, toxoplasmosis has moved with eutherian mammals to Australia and impacted marsupials (Daszak *et al.* 2000). Further, introduced avian pathogens including avian malaria caused extinctions of nearly half of the endemic land birds in Hawaii (van Riper *et al.* 1986).

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In the face of environmental change, species must either adapt, or become extinct

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Adaptation may take the form of either immediate physiological or behavioural modifications, where individuals change to cope with altered conditions, or genetic adaptation, where natural selection alters the genetic composition of populations over several or many generations.

Physiological adaptations by individuals (phenotypic plasticity) include modification in haemoglobin levels with altitude, immune responses against diseases, induction of enzymes to utilize altered diets, etc. Behavioural adaptations may include altered food preferences, predator avoidance behaviour, etc. There is however a limit to both physiological and behavioural adaptation. If environmental changes are greater than any individual can tolerate, then the species becomes extinct.

Evolutionary change through **natural selection** is the long-term genetic response to environmental change. This is referred to as **adaptive evolution**. When adaptive evolutionary changes continue over time, they may allow a population to prosper under conditions more extreme than any individual could originally tolerate.

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Adaptive evolutionary changes may allow populations to cope with conditions that no individual could previously survive

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Adaptive evolution is observed wherever large, genetically variable populations are subjected to altered biotic or physical environments. It is of major importance in eight conservation contexts:

- management to preserve the ability of species to evolve
- management of fragmented populations to minimize loss of adaptive evolutionary potential in small populations
- adaptation to marginal environments; most endangered species now exist only on the periphery of their historical range (Channell & Lomolino 2000), and therefore must adapt
- genetic adaptation to captivity and its deleterious effects on reintroduction success (Chapter 20)
- adaptation of translocated populations to their new environment (Chapter 17)
- ability of predatory and invasive species to adapt to tolerate biocides
- ability of invasive species to adapt to new locations
- crossing of populations adapted to different environments may reduce reproductive fitness, or allow adaptation to novel environments (Chapters 14 and 16).

This chapter provides evidence for the ubiquity of adaptive evolutionary change, considers the factors controlling the evolution of populations and examines the impact of selection on populations.

Adaptive evolutionary changes have been documented in animal morphology, behaviour, colour, host plant resistance, prey size, body size,

alcohol tolerance, life history attributes, disease resistance, predator avoidance, tolerance to pollutants, biocide resistance, etc. (Thompson 1998; Mousseau *et al.* 2000; Hoekstra 2006).

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Genetic adaptation of species to their environmental conditions is ubiquitous in species with genetic diversity

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Adaptive evolutionary changes in plants include those to soil conditions, water stress, flooding, light regimes, exposure to wind, grazing, air pollution and herbicides (Briggs & Walters 1997). Plants have evolved a wide range of secondary compounds to avoid being eaten by herbivores. For example, white clover and bird's-foot trefoil are polymorphic for the production of cyanogenic glucosides that provide protection from herbivory by slugs and snails (Briggs & Walters 1997). Plant populations adapted to diverse ecological conditions are so common that they have their own term (**ecotypes**).

Adaptive evolutionary changes have allowed species to inhabit almost every imaginable niche on Earth; altitudes from deep ocean trenches to 6500 m on Mount Everest, from arctic saline pools at  $-23^{\circ}\text{C}$  to boiling thermal springs and deep sea vents, from oceans and freshwater to deserts, while plants have adapted to grow in almost every soil on the planet (Dobzhansky *et al.* 1977).

---

Different species evolve similar characteristics when placed in similar environments – convergent evolution

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**Convergent evolution** of similar forms in equivalent ecological niches, but derived from different progenitors, illustrates the pervasive influence of adaptive evolution. For example, bats, birds and pterodactyls independently evolved flight, and there are morphologically similar forms amongst marsupials in Australia and eutherian mammals from other parts of the world, e.g. the marsupial versus eutherian wolves, moles and mice (Futuyma [1998](#)).

Measurable adaptive evolutionary changes have occurred from year to year in beak and body dimensions in Darwin's medium ground finch in response to environmental changes associated with El Niño–Southern Oscillation climate changes ([Chapter 5](#)). Rapid evolutionary changes in migration rates have been described in birds and plants (Berthold *et al.* [1992](#); Cody & Overton [1996](#)).

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Adaptive evolutionary changes may be rapid

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Palumbi ([2001](#)) has argued that humans are now the greatest evolutionary force. In many cases, adaptive evolutionary changes have been recorded in human-affected species. Rabbits in Australia evolved resistance to the myxoma virus when it was introduced as a control measure ([Box 6.1](#)). Over 200 species of moths worldwide exhibit industrial melanism in polluted industrial areas (Kettlewell [1973](#)). Several species of plants have evolved tolerance to heavy metals in the process of colonizing polluted heavy-metal mine wastes and almost 200 species of plants have evolved resistance to



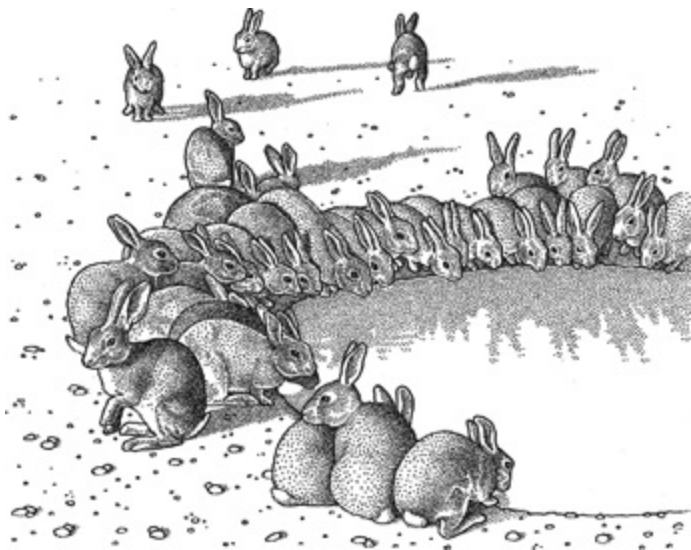
herbicides (Briggs & Walters 1997; Heap 2007). A population of a subspecies of checkerspot butterfly in Nevada has evolved altered host preference, now preferring an introduced weed (Singer *et al.* 1993).

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Evolutionary changes have been observed in response to many human-caused environmental changes

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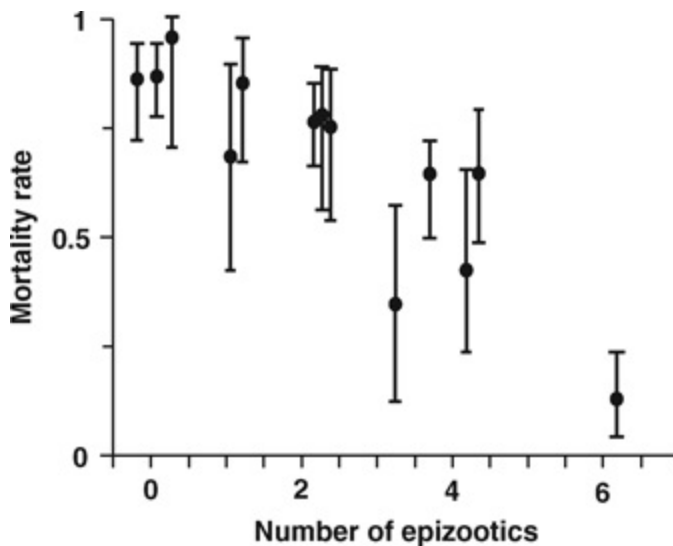
**Box 6.1 Rapid adaptive evolutionary changes in rabbits in Australia following the introduction of myxoma virus as a control agent (Fenner & Ratcliffe 1965; Nowak 2004)**



Following introduction of wild rabbits into Australia by European settlers in the nineteenth century for sport hunting, they rapidly reached plague proportions throughout much of the country. Rabbits caused many native plant species to decline and were one of the causes in the decline of

native marsupial bilbies. Attempts to control rabbits with poisons, warren ripping and many thousands of kilometres of rabbit-proof fences failed to halt their spread.

When myxoma virus was introduced into Australia in 1950, mortality rates of infected rabbits were over 99%. Strong directional selection resulted in rapid increases in genetic resistance of rabbits to the virus, mortality to a standard virus strain dropping from around 90% to 25% in 1958 (the sixth epizootic), as shown below.



Recently, callici virus escaped into the Australian rabbit population and adaptation to tolerate this virus is expected.

Many species have evolved resistance to biocontrol agents (insecticides, pesticides, antibiotics, etc.) (Georghiou [1986](#)). For example, hundreds of insect species have evolved resistance to insecticides (McKenzie [1996](#)) and microbes rapidly evolve resistance to each new antibiotic (Garrett [1994](#)). Rats and mice have evolved resistance to warfarin and other anticoagulant rodenticides (Futuyma [1998](#)).

Adaptive evolutionary changes as a result of climate change have already

been observed in red squirrels, blackcap birds, several species of fruit flies, pitcher plant mosquitoes and European beech trees. Sadly, in great tits and European beech the rate of adaptation is not keeping up with the pace of environmental change (Reusch & Wood [2007](#)).

## Factors controlling the evolution of populations

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Populations evolve through the action of selection, mutation, migration and chance

---

Our objective in conservation genetics is to preserve species as dynamic entities, capable of evolving to adapt with environmental changes. Consequently, we must appreciate how genetic diversity arises, what forms of genetic diversity exist and how it is lost.

Evolution involves a change in the genetic composition of a population. At its simplest level, this represents a change in the frequency of an allele due to mutation, selection, migration or chance.

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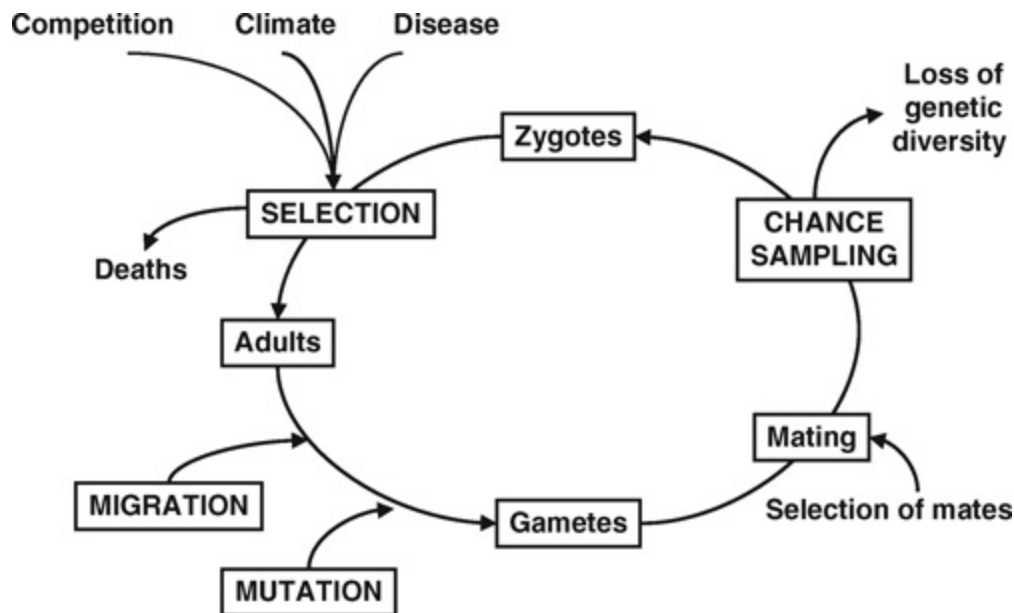
Evolution at its simplest level is a change in the frequency of an allele

---

The roles of these factors can be summarized as follows:

- mutation is the source of all genetic diversity, but is a very weak evolutionary force over the short term, as mutation rates are generally very low
- selection is the only force causing adaptive evolutionary change
- migration (gene flow) reduces differences among populations generated by mutation, selection and chance
- chance effects in small populations lead to loss of genetic diversity
- fragmentation and reduced migration limiting gene flow generate random differentiation among sub-populations derived from the same original source population.

These insights have been obtained principally from detailed studies of population models. An evolving population can be modelled as a complex system influenced by mutation, migration, selection and chance, operating within the context of the breeding system (Fig. 6.1). To evaluate the importance of the components of an evolving population, we model it with none of the factors operating, then with each of the factors individually, followed by two at a time, etc. By so doing we can estimate the impact of each factor and what role it is likely to play in evolution. Further, we can identify those circumstances where particular factors can be ignored, as it is rare for all factors to have significant effects simultaneously. For example, mutations occur at very low rates, and we can often ignore them over the short term.



**Fig. 6.1** An evolving population as a complex system.

In [Chapter 4](#) we showed that allele and genotype frequencies at an autosomal locus attain Hardy–Weinberg equilibrium after one generation of random mating in populations free from mutation, migration, selection or chance. In this chapter and the next we consider the independent action of selection, mutation and migration, followed by the joint actions of mutation with selection and migration with selection. Chance effects are generally minor in large populations, so we defer detailed treatment of them until [Chapter 8](#).

## Role of mathematical models

Simple mathematical models have a crucial role in illuminating genetics of the evolutionary process

To investigate the evolutionary impacts of selection, mutation, migration and chance we will build simple mathematical models. Wilson (1975) pointed out that such models can:

- establish quantitative laws to describe underlying processes
- provide testable predictions
- provide expressions for estimating parameters that are difficult or impossible to estimate otherwise
- predict the existence of still undiscovered phenomena and unexpected relations among phenomena.

The models we build are usually simplifications of the real world. To determine whether our models explain that world, we require quantitative predictions that can be evaluated against experimental or observational data. For example, equations to predict the decline in frequency of a recessive lethal allele are evaluated later in this chapter and applied to predict changes in the frequency of chondrodystrophy (a lethal dwarfism) in California condors. In Chapter 11, equations are presented that predict that equalizing family sizes effectively doubles the size of captive populations (and so maximizes the use of scarce captive breeding spaces).

Often methods for estimating parameters that we wish to know are not obvious. Parameters that have been estimated using models include:

- mutation rates in humans (from the balance between mutation and selection)
- magnitude of selection on industrial melanism in moths (from models of the time taken for changes in allele frequency)
- extent of gene flow among populations and species (from allele frequencies in different populations), including migration rates in human populations
- relative number and effect of deleterious alleles in populations of endangered species (from the effect of inbreeding on mortality).

By building mathematical models of evolutionary processes, new insights are often revealed. For example, cost–benefit analyses of altruistic behaviour led to the predictions that it would typically involve close relatives, and that it would be more common in haplo-diploid species such as the Hymenoptera than in diploids.

## Selection

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Selection is the only force that causes adaptive evolution

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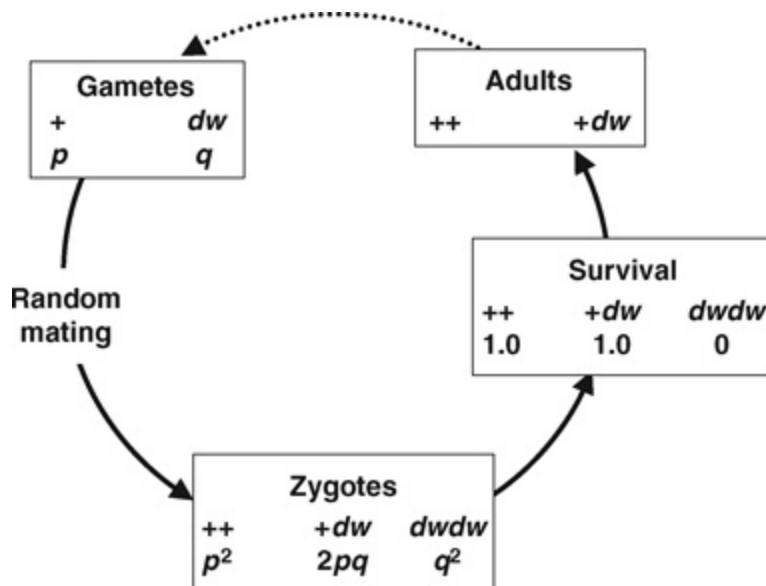
Alleles whose carriers have more fertile offspring surviving to reproductive age increase in frequency, while alleles whose carriers have fewer offspring decrease in frequency. Selection operates at all stages of the life cycle. In animals this involves acquisition of mates, mating ability and fertility of males and females, fertilization success of sperm and eggs, number of offspring per female, survival of offspring to reproductive age and longevity. In plants, selection can involve pollen production, ability of pollen to reach the stigma of flowers, germinate, grow down the style and fertilize, number of ova, viability of the fertilized zygotes, and their ability to disperse, germinate and grow to sexual maturity, and the fertility of the resulting plant. For simplicity, we will use models that involve differential survival of individuals from zygote formation to adult. The consequences of selection on other stages of the life cycle are similar.

## Recessive lethal

---

Selection reduces the frequency of deleterious alleles

To illustrate and model the action of natural selection we will first examine an extreme case, that of a recessive lethal, where all recessive homozygotes die but heterozygotes have normal survival. For example, all individuals homozygous for chondrodystrophic dwarfism ( $dwdw$ ) in endangered California condors die around hatching time. The flowchart for a simple model with discrete generations, random mating and viability selection against chondrodystrophy is shown in Fig. 6.2 and modelled in Table 6.1.



**Fig. 6.2** Selection model for a recessive lethal.

**Table 6.1** *Modelling the impact of selection against chondrodystrophy (a recessive lethal) in California condors*



	Phenotype			Total
	Normal	Normal	Dwarf	
	Genotype			
	++	+dw	dwdw	
Zygotic frequencies	$p^2$	$2pq$	$q^2$	1.0
Relative fitnesses	1	1	0 (lethal)	
After selection (freq. $\times$ fitness)	$p^2 \times 1$	$2pq \times 1$	$q^2 \times 0 = 0$	$1 - q^2$
Adjusted frequencies	$\frac{p^2}{1 - q^2}$	$\frac{2pq}{1 - q^2}$	0	1.0

The frequency of the  $dw$  allele in the next generation after selection ( $q_1$ ) is

$$q_1 = \frac{\text{homozygotes} + 1/2 \text{ heterozygotes}}{\text{total}} = \frac{0 + pq}{1 - q^2} = \frac{q(1 - q)}{(1 - q)(1 + q)}.$$

$$q_1 = \frac{q}{1 + q} \quad (6.1)$$

Note that  $1 - q$  can be substituted for  $p$ , as  $p + q = 1$ .

The change in frequency  $\Delta q$  is:

$$\Delta q = q_1 - q = \frac{q}{1 + q} - q = \frac{q - q(1 + q)}{1 + q}$$

$$\Delta q = \frac{-q^2}{1 + q} \quad (6.2)$$



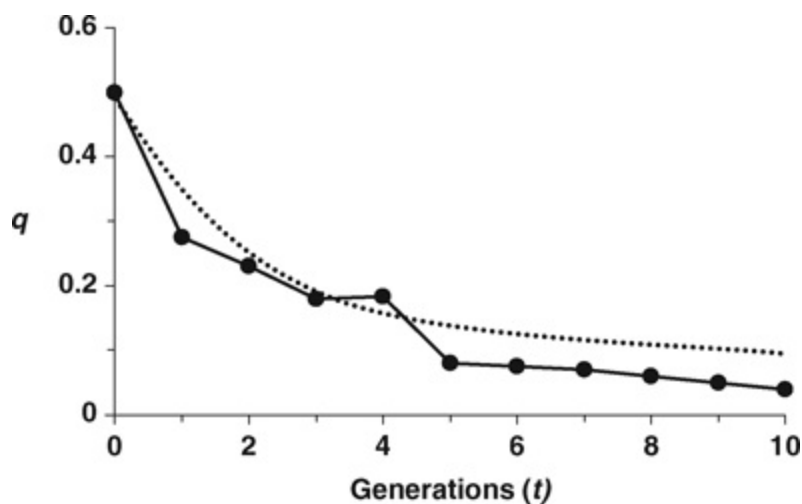
California condor

We begin with the normal allele (+) at a frequency of  $p$  and the recessive lethal ( $dw$ ) allele at a frequency of  $q$ . With random mating, the genotype frequencies at zygote formation are the Hardy–Weinberg equilibrium

frequencies  $p^2$ ,  $2pq$  and  $q^2$ . However, the three genotypes have different survival. It is the relative rather than the absolute survival of the three genotypes that determines the changes in allele frequencies. For example, if the  $++$ ,  $+dw$  and  $dwdw$  genotypes have 75%, 75% and 0% survival, the relative values 1, 1 and 0 determine the impact of selection; we term these values the **relative fitnesses**.

The frequency of surviving adults is obtained by multiplying the zygotic frequencies by the relative fitnesses. For example, the frequency of lethal homozygotes goes from  $q^2$  at fertilization to  $q^2 \times 0 = 0$  in adults. After selection, we have lost some of the population ( $-q^2$ ), so the total no longer adds to 1. We must therefore divide by the total ( $1 - q^2$ ) to regain relative frequencies, as shown.

The allele frequency in the succeeding generation is then obtained by determining the allele frequency in survivors using the allele counting method described in [Chapter 4](#). Equation 6.2 demonstrates that the lethal  $dw$  allele always declines in frequency, as the sign of the change in frequency ( $\Delta q$ ) is negative. Further, the rate of decline slows markedly at lower frequencies as it depends on the square of the frequency ([Fig. 6.3](#)). Example 6.1 uses Equation 6.1 to calculate the change in frequency of the  $dw$  allele in California condors.



**Fig. 6.3** Change in frequency of a recessive lethal allele ( $q$ ) over

generations in a fruit fly population. The solid line is the observed change and the dashed line is the decline predicted by Equation 6.1 (after Wallace 1963).

---

### **Example 6.1 Change in frequency of the chondrodystrophy allele in endangered California condors**

The expected frequency of the chondrodystrophy allele in adults as a result of natural selection can be predicted by using Equation 6.1 and substituting  $q = 0.17$  (initial frequency from Example 4.5), as follows:

$$q_1 = \frac{q}{1 + q} = \frac{0.17}{(1 + 0.17)} = 0.145$$

Thus, the frequency is expected to have dropped from 17% to 14.5% as a result of one generation of natural selection, a reduction of about 15%.

---

The observed frequency of a recessive lethal allele in an experimental fruit fly population declined continuously at approximately the rate predicted by Equation 6.1 with the decline slowing over time, as expected (Fig. 6.3). An important implication of this relationship is that it becomes progressively harder to reduce the frequency of a deleterious recessive allele as its frequency declines.

The observed decline is slightly faster than predicted, suggesting that heterozygotes have a slightly reduced fitness compared to homozygous normal individuals. ‘Recessive’ alleles frequently have a small impact in heterozygotes.

Selection not only applies to lethals. Any allele that changes the relative fitness of its carriers will be subject to selection. If the effect on fitness is small then the change in frequency will be correspondingly smaller. The methods for deriving allele frequency changes due to all other forms of selection are similar to that used above.

## Adaptive evolutionary change

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Selection increases the frequency of advantageous alleles

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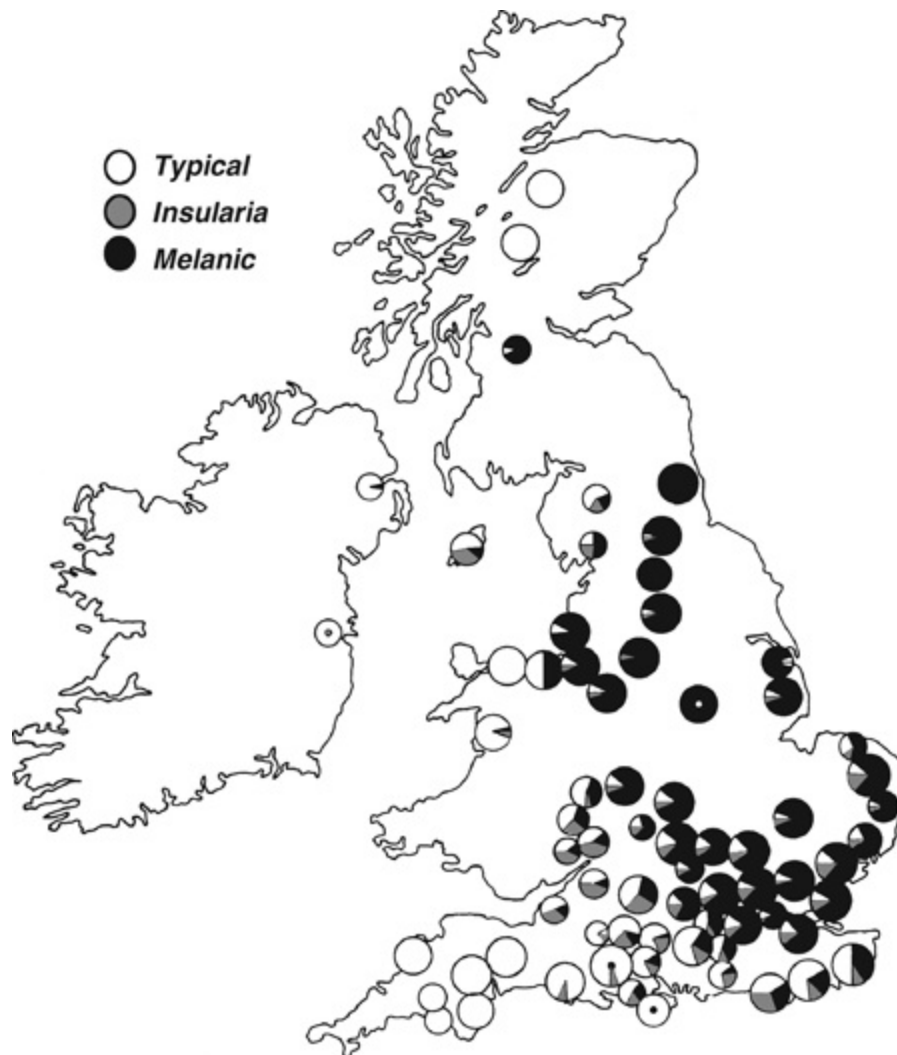
In conservation genetics, we are concerned both with selection against deleterious mutations (described above) and with selection favouring alleles that improve the ability of a population to adapt to changed environments. We will use industrial melanism in the peppered moth in Britain to illustrate adaptive evolutionary change (Kettlewell [1973](#); de Roode [2007](#)). Prior to the industrial revolution, the peppered moth was well camouflaged as it rested on speckled lichen-covered trees (chapter frontispiece). However, when sulphur pollution killed most lichen and soot darkened trees, the speckled moth became clearly visible. The previously rare dark variants (melanics) were better camouflaged on the black trees and suffered less predation, leading to higher frequencies of the melanic allele ( $M$ ) in the industrial areas, than in relatively unpolluted areas ([Box 6.2](#)). The melanic form of the peppered moth was first recorded in 1848, but by 1900 they represented about 99% of all moths in the polluted midlands of England.

---

**Box 6.2 Adaptive changes in the frequency of industrial melanism due to selection in polluted areas (after**

## Kettlewell 1973; de Roode 2007)

In the 1950s the melanic form of the peppered moths had high frequencies in industrial areas (Midlands, around London in the southeast and around Glasgow toward the northwest) and low frequencies in less polluted areas, as shown by the pie diagrams in the map of the UK below (Kettlewell 1973).



(Reprinted with permission from Macmillan Publishers Ltd. Kettlewell, H. B. D. 1958. A survey of the frequencies of *Biston betularia* (L.) (Lep.) and its melanic forms in Great Britain. *Heredity* 12: 551–572, copyright 1958.)

The melanic form of the moth is due to a single dominant allele. The predicted impacts of selection on the melanic and typical allele frequencies are given below. We begin with frequencies for melanic ( $M$ ) and typical ( $t$ ) (non-melanic) alleles of  $p$  and  $q$ , respectively, and assume that we are dealing with a large random mating population with no migration or mutation. Selection is assumed to occur on adults, but before reproduction. Since the  $tt$  genotype has poorer survival than melanics in polluted areas, but we do not know the precise value, we give it a relative fitness of  $1 - s$ , where  $s$  is the selection coefficient. The value of  $s$  represents the reduction in fitness of the  $tt$  genotype compared to the fittest genotypes,  $MM$  and  $Mt$ . For example, if the survival of  $tt$  was only 70% of that of  $MM$  and  $Mt$ , the selection coefficient is  $1 - 0.7 = 0.3$ .

	Melanic $MM$	Melanic $Mt$	Typical $tt$	Total
Zygotic frequencies	$p^2$	$2pq$	$q^2$	1.0
Relative fitnesses	1	1	$1 - s$	
After selection	$p^2$	$2pq$	$q^2(1 - s)$	$1 - sq^2$
Adjusted frequency	$\frac{p^2}{1 - sq^2}$	$\frac{2pq}{1 - sq^2}$	$\frac{q^2 - sq^2}{1 - sq^2}$	1.0

Frequency of  $M$  after selection ( $p_1$ ) is

$$\begin{aligned}
 p_1 &= \frac{p^2}{1 - sq^2} + \left(\frac{1}{2}\right) \frac{2pq}{1 - sq^2} \\
 &= \frac{p^2 + pq}{1 - sq^2} = \frac{p(p + q)}{1 - sq^2} \\
 &= \frac{p}{1 - sq^2}
 \end{aligned}$$

The change in frequency of  $M$  ( $\Delta p$ ) is

$$\begin{aligned}
 \Delta p &= p_1 - p \\
 &= \frac{p}{1 - sq^2} - p = \frac{p - p(1 - sq^2)}{1 - sq^2} \\
 &= \frac{spq^2}{1 - sq^2}
 \end{aligned}$$

Thus, the melanic allele increases in frequency, as the sign of  $\Delta p$  is positive. The rate of increase depends upon the selection coefficient and upon the allele frequencies.

If the melanic allele was at a frequency of  $p = 0.005$  in 1848, and typicals had only 70% the survival of melanics ( $s = 0.3$ ) in polluted areas, then the frequency of the melanic allele would change in one generation to

$$p_1 = \frac{p}{1 - sq^2} = \frac{0.005}{[1 - (0.3 \times 0.995^2)]} = 0.0071$$

The change in frequency is

$$\Delta p = p_1 - p = 0.0071 - 0.005 = 0.0021$$

Thus, the melanic allele increased by ~40% in the first generation.

---

The model of this selection developed in [Box 6.2](#) shows that the frequency of the  $M$  allele always increases until it is eventually fixed ( $p = 1$ ), as the sign of  $\Delta p$  is positive. The rate of change depends on the strength of selection against the non-melanic form ( $s$ , the **selection coefficient**) and the allele frequencies  $p$  and  $q$ .

Pollution controls would be expected to reverse the selective forces, and this has been observed. At a site near Liverpool, the frequency of melanics has dropped from 90% to 10% over the last 40 years. Declines have been observed in other areas of the UK, and parallel changes have occurred in the northern American sub-species of the peppered moth (Grant & Wiseman [2002](#); Cook *et al.* [2005](#)).

## Other selection models

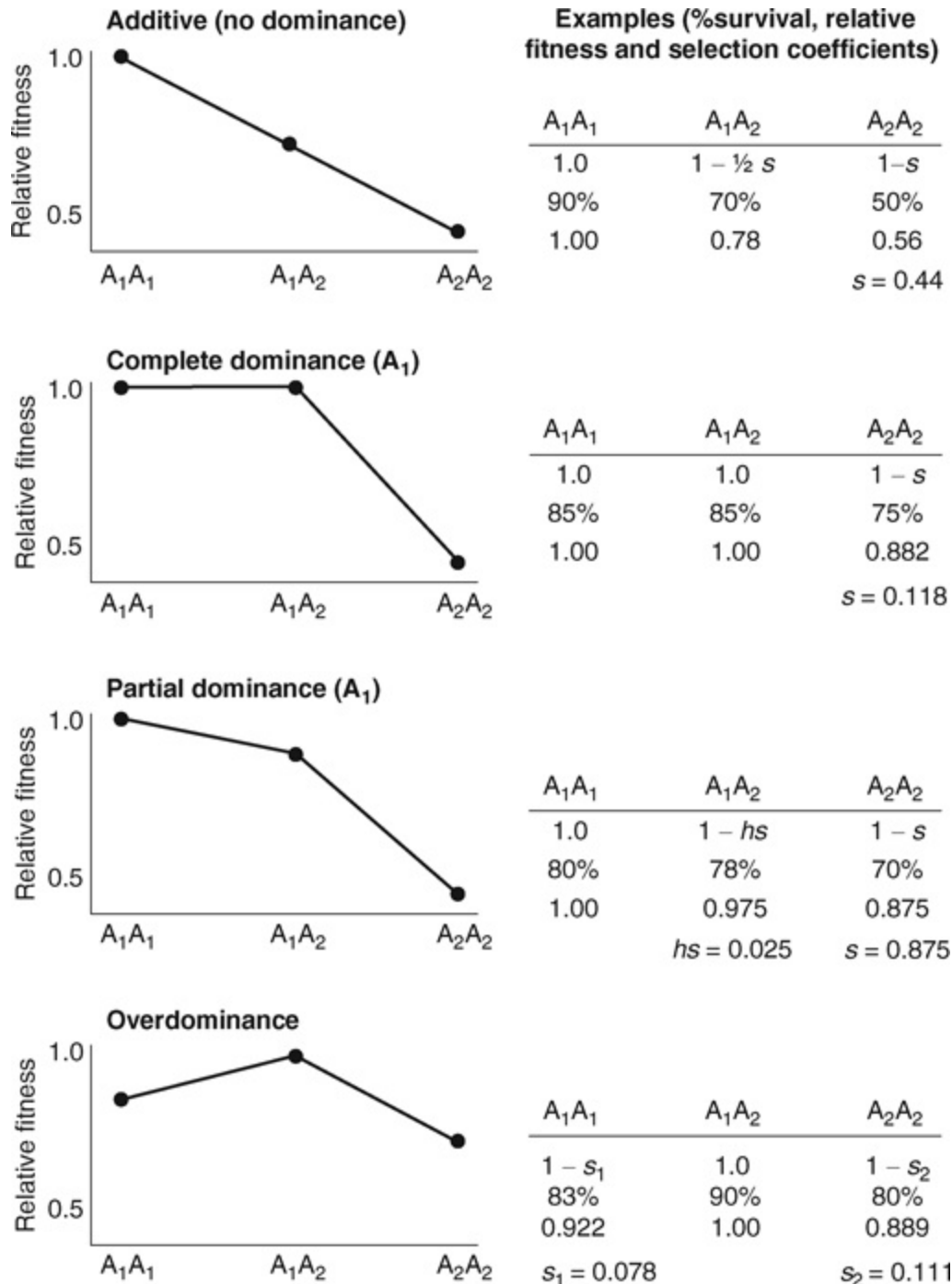
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The impact of selection depends on the strength of the selection, the mode of inheritance, and on the frequencies of the alleles

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Models with four different degrees of dominance with respect to fitness are illustrated in [Fig. 6.4](#). In each case, the selection coefficient ( $s$ ) represents the reduction in relative fitness of the genotype compared to that in the most fit genotype (fitness = 1). Values of  $s$  range from 0 to 1. In the **additive** case, the heterozygote has a fitness intermediate between the two homozygotes, while with **complete dominance** its fitness is equal to that of the  $A_1A_1$  homozygote. In the **partial dominance** case, the heterozygote has a fitness nearer one homozygote (here  $A_1A_1$ ) than the other, with its position on the scale depending on the value of  $h$ . In the overdominant case the heterozygote has a higher fitness than either homozygote.





**Fig. 6.4** Degrees of dominance with respect to fitness and examples of % survival, relative fitness and selection coefficients ( $s$ ) for each.

Selection coefficients are calculated from survivals of the genotypes by first converting the survivals into relative fitnesses. This is done by dividing all survival values by the highest value for the three genotypes. In the

additive example, the % survivals of 90, 70 and 50 are all divided by 90 to give relative fitnesses of  $90/90 = 1$ ,  $70/90 = 0.778$  and  $50/90 = 0.556$ , respectively. The relative fitness of  $A_2A_2$  (0.556) is equated to  $1 - s$ , giving an  $s$  value of 0.444.

The changes in allele frequency for different degrees of dominance with autosomal and sex-linked loci are shown in [Table 6.2](#). These are important in the contexts of adaptive changes due to selection, and of selection against deleterious alleles. Consequently, we will spend some time on the details of selection.

**Table 6.2** *Changes in allele frequency in one generation, with different dominance relationships for fitness for autosomal and sex-linked loci (initial frequencies are  $p$  and  $q$  for  $A_1$  and  $A_2$ , respectively)*

Initial frequencies and fitness of genotypes			Change in frequency															
<b>Autosomal</b>																		
$A_1A_1$ $p^2$	$A_1A_2$ $2pq$	$A_2A_2$ $q^2$	$\Delta q = q_1 - q_0$															
(a) Additive: selection against $A_2$																		
1	$1 - \frac{1}{2}s$	$1 - s$	$\frac{-\frac{1}{2}spq}{(1 - sq)}$															
(b) Complete dominance of $A_1$ , selection against $A_2$ ( $A_2$ recessive)																		
1	1	$1 - s$	$\frac{-spq^2}{(1 - sq^2)}$															
(c) Complete dominance of $A_1$ , selection against $A_1$ ( $A_2$ recessive)																		
$1 - s$	$1 - s$	1	$\frac{spq^2}{[1 - s(1 - q^2)]}$															
(d) Partial dominance of $A_1$ , selection against $A_2$																		
1	$1 - hs$	$1 - s$	$\frac{-spq[q + h(p - q)]}{(1 - 2hspq - sq^2)}$															
(e) Overdominance, selection against $A_1A_1$ and $A_2A_2$																		
$1 - s_1$	1	$1 - s_2$	$\frac{pq(s_1p - s_2q)}{(1 - s_1p^2 - s_2q^2)}$															
<b>Sex-linked<sup>a</sup></b>																		
<table> <tr> <th colspan="3">Females</th> <th colspan="2">Males</th> </tr> <tr> <td><math>X^{A_1}X^{A_1}</math></td> <td><math>X^{A_1}X^{A_2}</math></td> <td><math>X^{A_2}X^{A_2}</math></td> <td><math>X^{A_1}Y</math></td> <td><math>X^{A_2}Y</math></td> </tr> <tr> <td><math>p^2</math></td> <td><math>2pq</math></td> <td><math>q^2</math></td> <td><math>p</math></td> <td><math>q</math></td> </tr> </table>			Females			Males		$X^{A_1}X^{A_1}$	$X^{A_1}X^{A_2}$	$X^{A_2}X^{A_2}$	$X^{A_1}Y$	$X^{A_2}Y$	$p^2$	$2pq$	$q^2$	$p$	$q$	$\Delta q$
Females			Males															
$X^{A_1}X^{A_1}$	$X^{A_1}X^{A_2}$	$X^{A_2}X^{A_2}$	$X^{A_1}Y$	$X^{A_2}Y$														
$p^2$	$2pq$	$q^2$	$p$	$q$														
(f) Sex-linked recessive, selection against $X^{A_2}$																		
1	1	$1 - s$	1	$1 - s$														
(g) Additive sex-linked, selection against $X^{A_2}$																		
1	$1 - \frac{1}{2}s$	$1 - s$	1	$1 - s$														
<b>Haploid</b>																		
$A_1$	$A_2$																	
$p$	$q$																	
1	$1 - s$		$\frac{-spq}{1 - sq}$															

<sup>a</sup> For birds and Lepidoptera, the sexes must be reversed.

The effects of selection (change in allele frequency) for all the above models are all directly proportional to  $p$ ,  $q$  and  $s$  (Table 6.2). Response to selection for a quantitative character also depends upon  $pq$  (see Equation 5.5) and a measure of selection ( $S$ ) (Equation 5.7).

The change in allele frequency due to one generation of selection against a recessive  $A_2$  allele (Table 6.2, case b) is

$$\Delta q = \frac{-spq^2}{(1 - sq^2)} \quad (6.3)$$

Three important points are revealed by this equation:

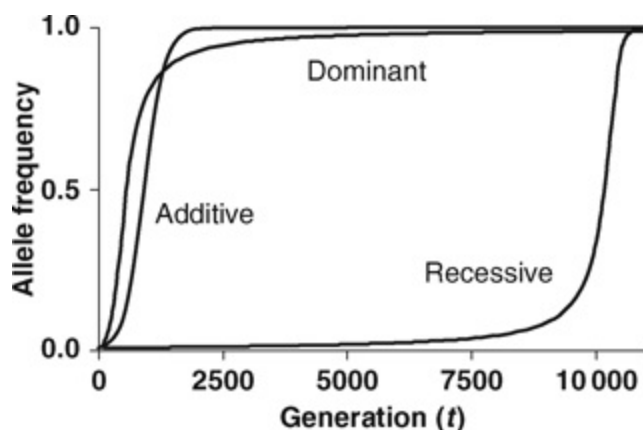
- the negative sign indicates that the deleterious  $A_2$  allele declines in frequency until it is completely eliminated
- the rate of change depends on the amount of selection ( $s$ )
- the rate of change depends on the allele frequencies,  $p$  and  $q$ .

Considering all the models in Table 6.2, the direction of change is always against the allele whose carriers have the lowest fitness. For example, the signs are opposite in direction for selection against  $A_2$  (case b in Table 6.2), compared to selection against  $A_1$  (case c). Heterozygote advantage (overdominance) is more complex, so we will defer treatment of it until Chapter 9.

The patterns of change in allele frequencies over time differ for dominant, additive and recessive alleles subject to the same selection coefficient and all beginning with the same frequency of the favoured allele (Fig. 6.5). The frequency of a favoured dominant allele initially increases more rapidly than does an additive, or recessive, allele. However, its rate of change slows markedly when it reaches a high frequency as the recessive homozygote, against which selection is acting, becomes increasingly rare. Conversely, the frequency of a favoured recessive allele initially increases very slowly, and

only after its frequency reaches ~10% does it rapidly rise in frequency to **fixation** (the population becomes homozygous and  $p = 1$ ). The dominant and recessive patterns are the mirror images of each other. The trajectory for the additive allele is initially slightly slower than that for the dominant, but it continues to increase and goes to fixation sooner.

For equivalent circumstances selection has greater impact on haploid loci than on autosomal diploid loci, with sex-linked loci intermediate



**Fig. 6.5** Change in frequency over generations for advantageous dominant, recessive and additive alleles. All have selective advantages of the beneficial homozygote of 1% over the deleterious one and all begin at a frequency  $p = 1\%$  (after Nei 1975).

Selection of the same intensity has a greater impact on a haploid locus than on an additive autosomal diploid locus, with an additive sex-linked locus intermediate, their  $\Delta q$  values for equivalent situations being in the ratios  $1 : 1/2 : 2/3$  (see Table 6.2). Further, selection against a recessive is much more effective for a sex-linked than an autosomal locus, due to highly efficient selection in haploid males. These observations become of major importance when we consider the balance between mutation and selection in the next

chapter.

## Number of generations required for a given change in allele frequency

---

The number of generations for a given change in frequency depends upon the selection coefficient, the mode of inheritance and the dominance of the allele

---

For a recessive lethal, an expression for the time in generations taken for a given frequency change can be obtained by rearranging Equation 6.1. If we refer to the allele frequencies after 0, 1, 2, and  $t$  generations as  $q_0$ ,  $q_1$ ,  $q_2$ ,  $q_t$  then

$$q_1 = \frac{q_0}{(1 + q_0)}$$

and

$$q_2 = \frac{q_1}{(1 + q_1)}$$

By substituting for  $q_1$ , and simplifying,

$$q_2 = \frac{q_0}{(1 + 2q_0)}$$

By extension:

$$q_t = \frac{q_0}{(1 + tq_0)}$$

(6.4)

By rearranging this expression we obtain the number of generations,  $t$ , required to change the allele frequency from  $q_0$  to  $q_t$ :

$$t = \frac{(q_0 - q_t)}{q_0 q_t} = \frac{1}{q_t} - \frac{1}{q_0} \quad (6.5)$$

Using this equation, we predict that it will take 94 generations to reduce the frequency of the chondrodystrophy allele in California condors from 17% to 1% (Example 6.2).

When selection is less intense, the time taken for a given change in allele frequency is longer. Equations for the number of generations required for allele frequency change are also available for the other models, but calculus is used in the derivations (Crow & Kimura 1970). They also depend upon the dominance relationships at the locus, as is evident from Fig. 6.5.

---

**Example 6.2 How long will it take to reduce the frequency of the recessive lethal chondrodystrophy allele in California condors to a frequency of 1%?**

To determine the number of generations required to reduce the frequency of the recessive lethal chondrodystrophy allele from 0.17 to 0.01, we use Equation 6.5 and substitute 0.17 for  $q_0$  and 0.01 for  $q_t$ , as follows:

$$t = \frac{1}{q_t} - \frac{1}{q_0} = \frac{1}{0.01} - \frac{1}{0.17} = 100 - 5.9 = 94.1$$

Consequently, it will take 94 generations to reduce the frequency of the chondrodystrophy allele to 1% as a result of natural selection.


---

## Selection coefficients

Expressions for change in allele frequencies ( $\Delta q$ ) can be used (after manipulation) to estimate selection coefficients. For example, Haldane (1924) estimated that typical peppered moths had a relative fitness about 1/3 lower than melanics in polluted environments, a value similar to that found in subsequent experiments by Kettlewell (Box 6.3).

### Box 6.3 Magnitude of selection involved in industrial melanism in the peppered moth (after Haldane 1924 and Kettlewell 1973)

The melanic form of peppered moths increased from a frequency of about 1% to about 99% in 52 years in the polluted midlands of England. Haldane (1924) used the following reasoning to derive an approximate expression for the time in generations for a dominant favourable ( $M$ ) allele to increase in frequency from  $p_0$  to  $p_t$ :

	Melanic $MM$	Melanic $Mt$	Typical $tt$	Total
Zygotic frequencies	$p^2$	$2pq$	$q^2$	1.0
Relative fitnesses	1	1	$1 - s$	
				
Frequencies 1848	0.01		0.99	$p_0 = 0.005$
Frequencies 1900	0.99		0.01	$p_t = 0.90$

The value for  $q_0$  is obtained by equating the typical frequency in 1848 (0.99) to  $q_0^2$ , taking square roots to obtain  $q_0 = 0.995$ , and equating  $p_0 = 1 - q_0 = 1 - 0.995 = 0.005$ .

Similarly,  $p_t$  is obtained by equating  $q_t^2 = 0.01$ , taking square roots to obtain  $q_t = 0.1$ , and equating  $p_t = 1 - q_t = 1 - 0.1 = 0.9$ .



The equation for change in frequency of the melanic allele (Box 6.2)

$$\Delta p = \frac{spq^2}{(1 - sq^2)} \sim spq^2$$

can be equated, using calculus, to the rate of change in allele frequency with time ( $dp / dt$ ) and integrated to obtain an expression for number of generations  $t$ :

$$t = \frac{1}{s} \left\{ \ln \left[ \frac{p_t(1 - p_0)}{p_0(1 - p_t)} \right] + \frac{1}{1 - p_t} - \frac{1}{1 - p_0} \right\}$$

As peppered moths breed annually,  $t = 52$  generations. Consequently, the selection coefficient is

$$s = \frac{1}{52} \left\{ \ln \left[ \frac{0.90 \times 0.995}{0.005 \times 0.10} \right] + \frac{1}{0.100} - \frac{1}{0.995} \right\}$$

$\therefore s = 0.32$

i.e. the survival of typical moths was predicted to be 32% lower in each generation than that of melanic moths in polluted areas. This estimate was ridiculed at the time.

Thirty years later, experiments done by Kettlewell yielded a selection coefficient of about this magnitude, as shown below. Kettlewell captured melanic and typical moths, marked and re-released them. A second sample of moths was captured shortly afterwards. The results were as follows:

	Polluted area		Unpolluted	
	Melanic	Typical	Melanic	Typical
Numbers marked and released	447	137	473	496
Released moths recaptured	27.5%	13.1%	6.34%	12.5%

The recapture rate for melanics was approximately twice as high as that for typicals in the polluted area, yielding a selection coefficient of about 50%. Selection in unpolluted areas was approximately as strong in the opposite direction. The selection coefficient is higher than predicted by

Haldane, attributable both to the use of a very heavily polluted area to carry out the experiment, and to migration of moths from unpolluted areas.

Selection coefficients for morphological characters may be strong, especially for polymorphisms involved in camouflage. However, selection on protein and DNA polymorphism is thought to be very much weaker, around 1% or less. For example, selection coefficients are typically only 1% or less at the MHC, where evidence of selection is compelling (Satta *et al.* 1994). Many polymorphisms at the DNA level may involve no selective differences, or very weak selection. It is a matter of debate as to whether allozyme polymorphisms are neutral, or subject to weak selection (Chapter 9).

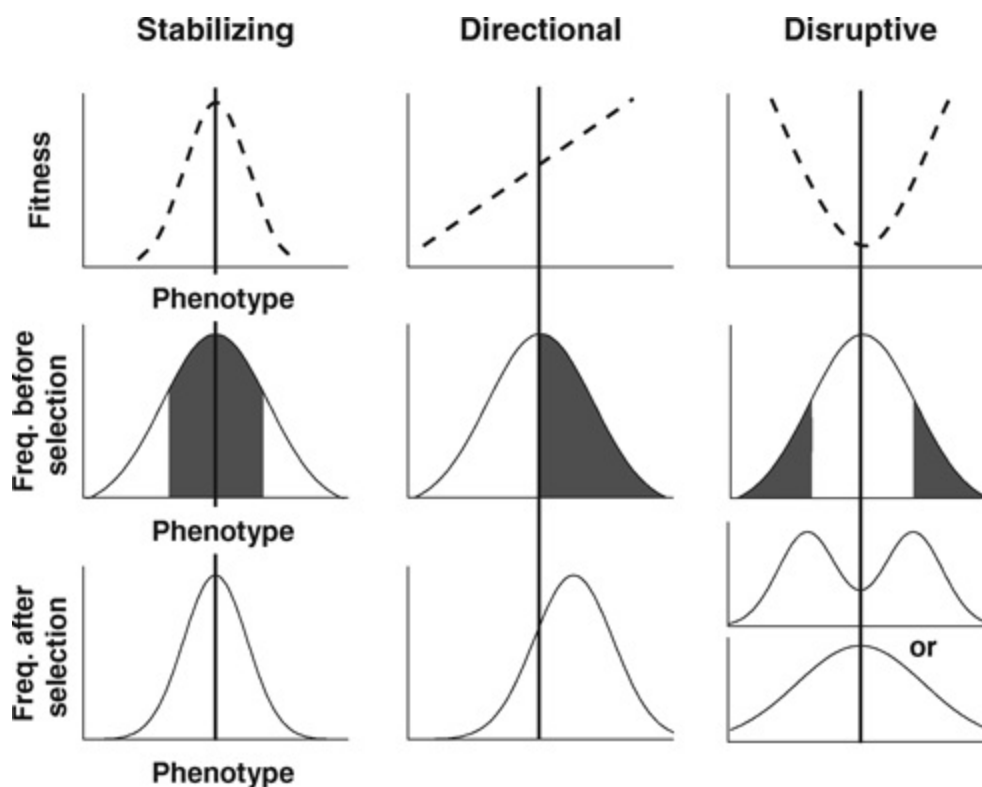
## Selection on quantitative characters

Adaptive evolutionary change results from natural selection on reproductive fitness, a quantitative character

So far we have discussed the effects of selection on allele frequencies at single loci. For quantitative characters, the primary subject of evolution, variation is determined by segregation at multiple loci in combination with varying inputs from environmental effects (Chapter 5). Consequently, we now switch from discussing the effects of selection on allele frequencies to the effects of selection on phenotypic means and variances.

Natural selection on quantitative characters affects the character mean and/or variance

There are three basic forms of selection that operate on quantitative characters: directional, stabilizing and disruptive ([Fig. 6.6](#)). **Directional selection** favours phenotypes towards one end of the distribution and results in a shift in the mean in this direction (provided there is genetic variation) ([Chapter 5](#)). **Stabilizing selection** favours phenotypic intermediates and results in no change in the mean, but may result in reduced variation in future generations. **Disruptive selection** favours both phenotypic extremes and does not alter the mean, but may lead to increased variation in future generations.



**Fig. 6.6** Forms of selection operating on quantitative characters (after Futuyma [1979](#)). Stabilizing, directional and disruptive selection are shown. The upper panels indicate the relationships between phenotype and fitness,

the middle panels the phenotypic distributions before selection and the bottom panels the distributions after selection in the subsequent generation. The shaded areas in the middle panels indicate the portions of the distribution favoured by selection.

In a constant and relatively uniform environment, reproductive fitness is subject to directional selection, while characters more peripheral to reproduction and survival typically exhibit selection favouring phenotypic intermediates (stabilizing selection). In heterogeneous environments, selection may vary in different directions in different habitats (disruptive). A review of phenotypic selection in natural populations indicates that strong directional selection is uncommon, and that stabilizing selection is neither stronger nor more common than disruptive selection (Kingsolver *et al.* [2001](#)). We elaborate on these three forms of selection below.

## Directional selection

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Consistent selection in the one direction (directional selection) results in phenotypic change in that direction, provided there is genetic diversity

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Directional selection occurs when the environment is changing in a consistent manner such as global warming, increasing levels of pollutants in air, water or soil, or when new diseases affect a population. Directional selection has been shown to produce large changes in outbred populations of many species of wild and domestic animals and plants, including changes in reproductive rate, growth rate in body size, behaviour, chemical composition, tolerance to heavy metals and resistance to disease (Frankham & Weber [2000](#)). For example, selection for tameness changed silver foxes from extremely hostile aggressive animals to ones approaching the tameness of

domestic dogs within 17 generations. Trophy hunting in wild populations of bighorn sheep in Canada that selectively removed rams with the largest horns resulted in genetic reductions in horn size and body size (Coltman *et al.* 2003).

Reproductive fitness in a relatively constant environment is subject to directional selection; the individuals with the highest fitness have the most offspring surviving to reproductive maturity and make greatest genetic contributions to the next generation.

Adaptive evolutionary changes in populations brought into captivity may occur very rapidly. For example, wild rats maintained in captivity for 25 generations under ‘natural’ selection increased in reproductive fitness in the captive environment by more than three-fold (King 1939), while large white butterflies in captivity increased in fecundity by 13-fold over 100–150 generations (Lewis & Thomas 2001). Captive populations of endangered species are likely to show similar adaptations. Disturbingly, this will typically result in decreased fitness when they are reintroduced into the wild (see Chapter 20).

Adaptive evolutionary change may utilize either pre-existing genetic diversity, or that arising due to mutation. Most adaptation in the short to medium term in higher eukaryotes is due to pre-existing genetic diversity, since there may be a long waiting time for beneficial mutations to arise in any but vast populations (Hartley *et al.* 2006; Chapter 7). Plant species that evolved heavy-metal tolerance and colonized polluted mine tailings in Great Britain were those with pre-existing genetic variation for heavy-metal tolerance, while species without the prerequisite genetic variation did not evolve resistance (Bradshaw 1991). The American chestnut has been driven almost to extinction by the introduced chestnut blight disease, presumably because it did not have genetic diversity for resistance and no disease resistant mutations have occurred since (Burdon 1987). In contrast, microbe populations can adapt rapidly since they are so vast that new mutations occur at most loci in every generation.

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Selection response in the short term operates primarily on pre-existing genetic diversity, rather than on new mutations, as mutation is a rare event

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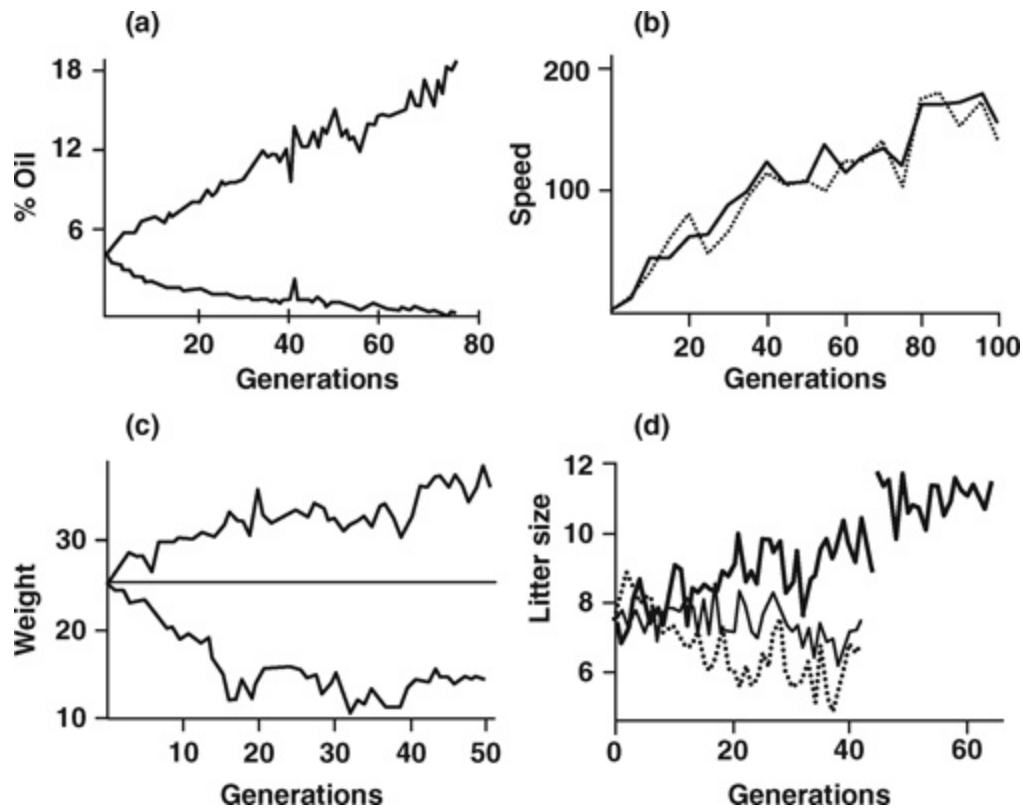
## Long-term adaptive changes

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Directional selection for many generations in large populations may produce extremely large genetic changes

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Large changes in phenotype over time have been observed in many populations subjected to directional selection, especially in laboratory and domestic species ([Fig. 6.7](#)). One of the most rapid and sustained responses to selection was an 85-fold change for flying speed over 100 generations in fruit flies ([Fig. 6.7b](#)).



**Fig. 6.7** Long-term response to directional selection. (a) Two-way selection for oil content in maize seeds (after Dudley 1977). (b) Selection for increased flying speed in replicate populations of fruit flies (Weber 1996). (c) Two-way selection for six-week body weight in mice (after Roberts 1966). (d) Selection for litter size in mice (after Falconer 1977); the bold solid line shows the impacts of selection for increased litter size, the dotted line selection for decreased litter size, while the fine solid line is an unselected control population.

In the fossil record, the height of the molar tooth in the horse lineage increased from ~4.7 mm to 52.5 mm over about 40 million years, presumably as an adaptive change from browsing on leaves and succulent plants to feeding on grass, which contains silica and wears teeth rapidly (Manly 1985; Futuyma 1998). It only requires selective deaths of about one individual in a million per generation to account for this evolutionary change. Brain size increased markedly in the human lineage from about 400 cm<sup>3</sup> to 1400 cm<sup>3</sup> in 3 million years (Futuyma 1998). The Haast eagle of New Zealand has increased in size by 10-fold in about 1 million years (Bunce *et al.* 2005).

Changes per unit time wrought by artificial selection are typically greater than those found in either the fossil record or in species colonizing new regions within recent history (Futuyma [1998](#)).

## Stabilizing selection

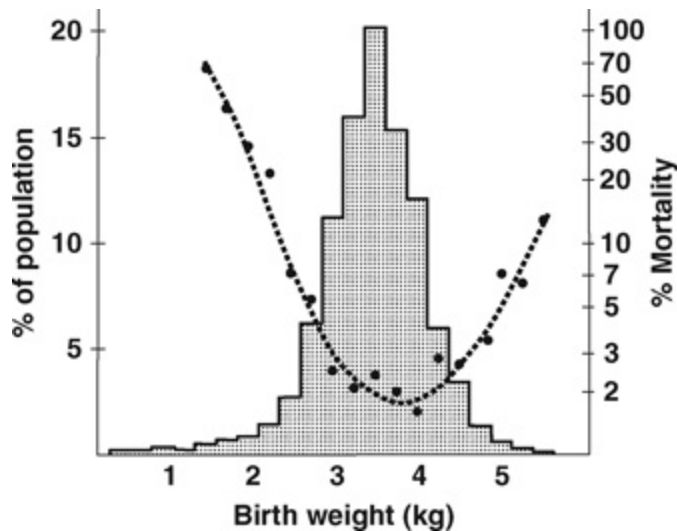
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Most peripheral quantitative characters in stable environments are subject to selection that favours phenotypic intermediates (stabilizing selection)

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Stabilizing selection is the most frequent form of selection found for peripheral quantitative characters (those weakly related to reproductive fitness) in populations in relatively uniform habitats. For example, stabilizing selection is found for birth weight in humans, with the smallest and largest babies having poorer survival than intermediate-weight babies ([Fig. 6.8](#)). Large babies are more likely to die from birth complications, while small babies are often insufficiently mature to survive (these effects are now greatly reduced by medical intervention). Similarly, stabilizing selection has been found for egg size in ducks and domestic fowl, for shell size in snails, body size in lizards, and for body dimensions in female sparrows following a severe storm (Lerner [1954](#)).





**Fig. 6.8** Stabilizing selection for birth weight in humans (after Mather 1973).

As stabilizing selection favours phenotypic intermediates, it is not expected to change the mean of the character. In its simplest form, stabilizing selection is expected to reduce genetic diversity (Roff 1997). However, selection favouring heterozygotes also causes phenotypic stabilizing selection, leading to the retention of genetic diversity (Robertson 1956; Chapter 9).

## Disruptive selection

When the environment is heterogeneous in space, selection may act in different directions in dissimilar habitats (disruptive selection)

Populations of conservation concern are often fragmented. If different fragments have dissimilar environmental conditions, natural selection will operate in diverse directions across fragments (Rueffler *et al.* 2006). For example, selection favours genotypes that tolerate heavy metals in several

grass species on polluted mine tailings in Wales, but operates against them in nearby non-contaminated pastures (Bradshaw & McNeilly 1981; Box 7.3).

The overall consequence of disruptive selection in fragmented habitats is generation of local adaptation. One possible long-term outcome of disruptive selection is speciation (Chapter 16).

## Summary

1. Change is a ubiquitous feature of the environmental conditions faced by species.
2. Species must evolve if they are to survive environmental changes that are more severe than individuals can cope with behaviourally or physiologically.
3. Adaptive evolution occurs through natural selection acting on genetic diversity within populations. At its simplest level it represents the change in frequency of a beneficial allele.
4. The impact of selection on a single locus depends upon selection coefficients, allele frequencies, dominance and the mode of inheritance.

## Further reading

Bradshaw & Holzapfel (2006) Brief review of adaptive evolutionary changes in a range of species as a result of global climate change.

Briggs & Walters (1997) *Plant Variation and Evolution*. Reviews evidence for adaptive genetic changes in plants.

Crow & Kimura (1970) *Introduction to Population Genetics Theory*. Classic reference for theoretical population genetics. More mathematical and advanced than this text.

Falconer & Mackay (1996) *Introduction to Quantitative Genetics*. Provides a very clear treatment of the topics in this chapter.

Hedrick (2005a) *Genetics of Populations*. Has a more extensive treatment of

many of the topics in this chapter.

Mousseau *et al.* (2000) *Adaptive Genetic Variation in the Wild*. Recent reviews on natural selection and adaptation in the wild.

Reusch & Wood (2007) Recent review of adaptive genetic changes in response to global climate change.

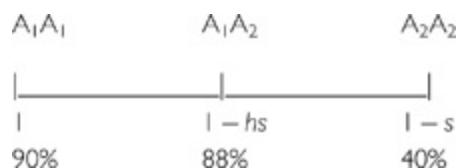
## Software

SELECTION: Free software to simulate the effects of selection, drift, mutation and migration on a single locus. [www.gsoftnet.us/GSoft.html](http://www.gsoftnet.us/GSoft.html)

WINPOP 2.5: Free software to simulate selection, drift, gene flow and migration. [www.genedrift.org/winpop.php/](http://www.genedrift.org/winpop.php/)

## Problems

- 6.1 Evolution. What is the only process that explains genetic adaptation of species to their environments?
- 6.2 Selection coefficients. If the three genotypes at a locus showing partial dominance have survival rates as shown, determine the relative fitnesses and the value of  $s$  and  $hs$ .



- 6.3 Selection. What will be the frequency of the chondrodystrophy allele (recessive lethal) in endangered California condors in the next three generations as a result of natural selection, if the initial frequency is 14.5% (Example 6.1)?
- 6.4 Selection. How many generations will it take for the frequency of chondrodystrophy (recessive lethal) to drop from 17% to 0.1% as a result of natural selection?
- 6.5 Selection. For a dominant allele ( $A$ ) with the relative fitnesses below

and an initial frequency  $p$  of 0.3, follow the genotype frequencies through from the zygotic stage, through selection, to the adjusted frequencies. Compute the new frequency of the allele after selection  $p_1$  and the change in frequency  $\Delta p$ .

	Genotypes			Total
	AA	Aa	aa	
Genotype frequencies at fertilization				1.0
Relative fitnesses	1	1	0.9	
After selection				
Adjust so total is 1				
New frequency of A = $p_1$ =				
Change in frequency $\Delta p = p_1 - p_0$ =				

- 6.6 Selection.** Derive the expression for the change in frequency as a result of selection against an additive deleterious allele, given that the initial frequency of the deleterious allele is  $q$  and the relative fitnesses are as shown in the table below.

	Genotypes			Total
	AA	Aa	aa	
Genotype frequencies at fertilization				1.0
Relative fitnesses	1	$1 - \frac{1}{2}s$	$1 - s$	
After selection				
Adjust so total is 1				
New frequency of A = $p_1$ =				
Change in frequency $\Delta p = p_1 - p_0$ =				

- 6.7 Impact of selection.** Assume that we are dealing with an allele that was favoured in the wild, but is deleterious in captivity. By how much would the frequency of the allele change if its initial frequency was 0.9 and was selected against with a selection coefficient of 0.1, as (a) additive, (b) recessive, (c) dominant and (d) partial recessive with  $hs = 0.02$ ?

## Practical exercises: Computer simulations

Use a computer simulation program such as SELECTION to simulate the following:

1. Selection against chondrodystrophy (recessive lethal) in California condors.

The relative fitnesses of the three genotypes at this locus are

$++$	$+dw$	$dwdw$
1	1	0

Commence with a frequency of the  $dw$  allele of 0.17 and simulate 100 generations of selection against this condition. What do you observe?

2. Selection for industrial melanism in peppered moths (favoured dominant allele). Industrial melanism increased in frequency from an allele frequency of about 0.005 in 1848 to about 0.90 in 1900 (52 generations). By trial and error, find a value of  $s$  that is adequate to explain this evolutionary event (e.g. try a relative fitness for  $tt$  of 0.5, and subsequently try other fitness values for  $t$ ). The selection model is

$tt$	$Mt$	$MM$
$1-s$	1	1

3. The impact of different selection coefficients on change in frequency of an advantageous additive allele. Compare the allele frequency trajectories with selection coefficients of 0.4, 0.1 and 0.01. Begin all cases with an initial frequency of 0.01. How long does each take to go to fixation? You will need to run the simulations for 1000 generations.

$aa$	$Aa$	$AA$
$1-s$	$1-\frac{1}{2}s$	1

What is the effect of different sized selection coefficients on the rate of allele frequency change?

4. Effect of dominance on allele frequency changes. Compare the allele frequency trajectories for advantageous dominant, additive and recessive alleles for loci with the following relative fitnesses. In each case begin with a frequency of 0.01 for the favoured allele and run for 2000 generations.

	aa	Aa	AA
Dominant	0.9	1	1
Additive	0.9	0.95	1
Recessive	0.9	0.9	1

How do they differ?

What difference does it make to the recessive case if it is partially recessive with a slight effect in the heterozygote (try a heterozygote fitness of 0.902).

5. Evolution of resistance to a biocide in a pest species. Predation by European red foxes is a major factor threatening small mammals in Australia. Sodium fluoroacetate poison (1080) is widely used to control foxes. If a dominant allele for 1080 resistance exists with an initial frequency of 0.1%, how long will it take for it to rise in frequency to 50% in a region with continuous baiting with 1080 (and no migration)? Assume that the relative fitnesses of RR, RS and SS under baiting are 1, 1 and 0.5, respectively.

## **Chapter 7 Evolutionary impacts of mutation and migration, and their interactions with selection in large populations**

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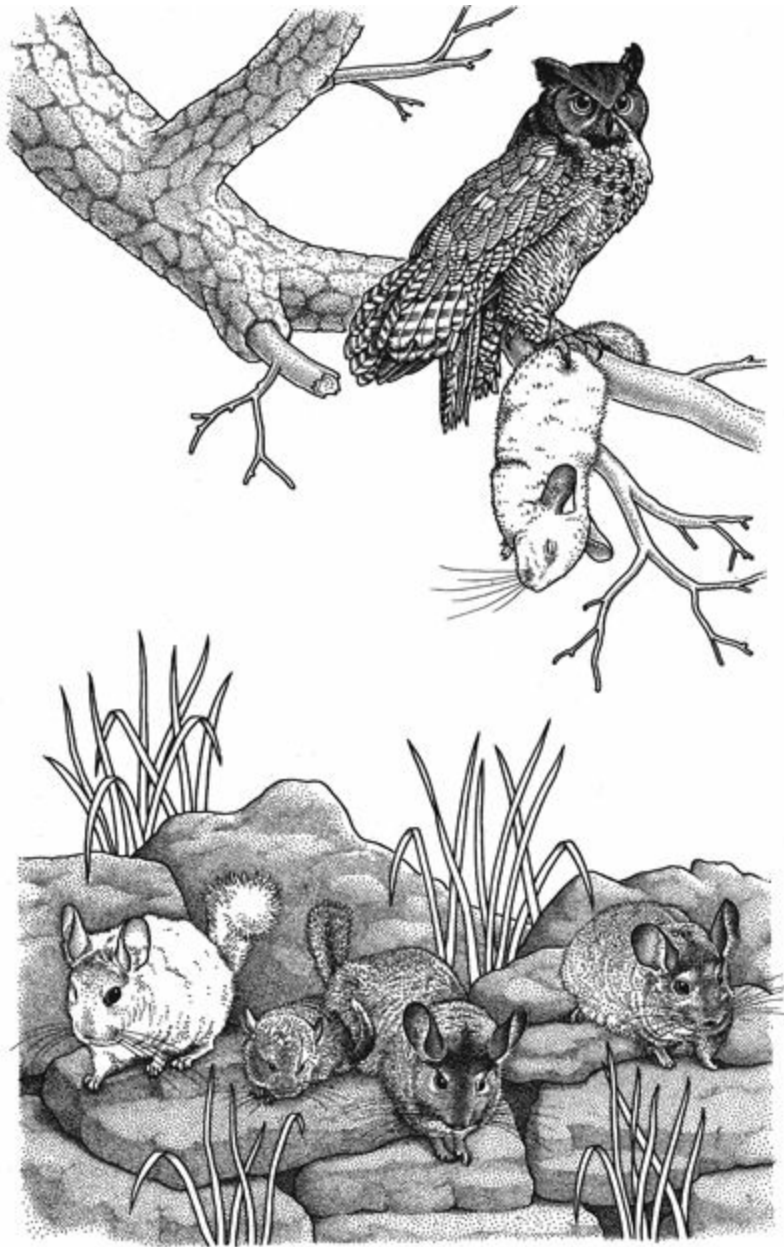
Mutation and migration are the only mechanisms for regaining lost genetic diversity. The balance between mutation and selection results in a 'load' of rare deleterious alleles that can lead to inbreeding depression

### **Terms**

Cline, gene flow, genetic load, introgression, migration, mutation, mutation load, mutation–selection balance, neutral mutation, silent substitutions, stable equilibrium, transposon

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Mutation–selection balance: white mutant chinchilla in South America being taken by a great horned owl, while camouflaged agouti chinchillas survive



## Factors controlling the evolution of populations

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Populations evolve through the action of mutation, migration, selection and chance

An evolving population is a complex system influenced by mutation, migration, selection and chance, operating within the context of the breeding system. In this chapter we consider mutation and migration, and the joint actions of these with selection, again in the context of large populations. These are essentially deterministic forces where the responses of large populations are predictable and different replicate populations subject to the same conditions will behave similarly.

### Importance of mutation and migration and their interactions with selection in conservation

Mutation and migration, and their interactions with selection have six important implications in conservation genetics:

- genetic diversity, lost by chance and selection, regenerates through mutation, but the rate is very slow
- when genetic diversity is lost in small threatened populations, it can be recovered immediately by migration from other genetically distinct populations; this is an extremely important genetic management tool for fragmented populations ([Chapters 14 and 17](#))
- migration usually reverses inbreeding depression ([Chapter 13](#))
- many rare species are being ‘hybridized out of existence’ by crossing with common related species ([Chapter 18](#))

- mutation and migration are usually important factors in the maintenance of genetic diversity ([Chapter 9](#))
- the balance between deleterious mutation and selection results in a pool of rare deleterious mutations (**mutation load**) in populations; inbreeding exposes these mutations, resulting in reduced reproduction and survival and increased extinction risk ([Chapters 12](#) and [13](#)).

## Origin and regeneration of genetic diversity

Genetic diversity is the raw material required for adaptive evolutionary change. While large populations of most naturally outbreeding species carry a substantial store of genetic diversity, it is lost by chance in small populations. This leads to the questions:

- how is genetic diversity produced?
- how quickly can it be regenerated?

Below we consider these questions.

## Mutation

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Mutation is the ultimate source of all genetic diversity

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A **mutation** is a sudden genetic change in an allele or chromosome. All genetic diversity originates from mutation. DNA is under constant assault by oxygen, radiation, various chemicals and even our own cellular processes. DNA repair processes have evolved to repair such damage, to minimize (but not eliminate) mutations and to provide protection against cancer and ageing

(Fuss & Cooper [2006](#)).

In this chapter we concentrate on single-locus mutations. They are generated by base substitutions, additions and deletions in the DNA, by gene duplications and by insertions and excisions of mobile segments of DNA (**transposons**). Half or more of the spontaneous mutations in fruit flies are due to transposable elements, but they are responsible for less than 1% of human mutations (Biémont & Vieira [2006](#)). Chromosomal mutations (duplications, deletions, inversions, translocations and polyploidy) are important in tracing speciation, and in hybrid infertility, so we shall defer consideration of them until [Chapter 16](#).

In the context of conservation, the most important mutations are those at loci affecting fitness traits, most notably lethal or deleterious mutations. Some mutations are beneficial ([Chapter 6](#)). However, many mutations that occur in non-coding regions of the genome and those that do not result in amino acid substitutions in proteins (**silent substitutions**) often have little or no impact on fitness (**neutral mutations**). Neutral mutations are, however, important as molecular markers and clocks that provide valuable information on genetic differences among individuals, populations and species.

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Mutations typically occur at a very low rate

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For a range of loci in eukaryotic species, the typical spontaneous rate of morphological mutations is one new mutation per locus per 100 000 gametes per generation ([Table 7.1](#)). Mutation rates are fairly similar across all eukaryotes, apart from microsatellites and mtDNA. Mitochondrial DNA in

animals has a much higher mutation rate than nuclear loci, making it a valuable tool in studying short-term evolutionary processes ([Chapter 20](#)).

**Table 7.1** *Spontaneous mutation rates for different loci and characters in a variety of eukaryotic species. Approximate mean rates are given as the frequency of new mutations per locus, per generation, except where specified otherwise*

Type of mutation	Rate per generation
Morphological mutations	
Mice, maize and fruit flies (normal → mutant)	$\sim 1 \times 10^{-5}$ / locus
Reverse mutation (mutant → normal)	$0.3 \times 10^{-5}$ / locus
Allozyme loci (mobility change)	$0.1 \times 10^{-5}$ / locus
Microsatellites	
Mammals	$1 \times 10^{-4}$ / locus
Fruit flies	$0.7 \times 10^{-5}$ / locus
DNA nucleotides	$10^{-8} - 10^{-9}$ / base / year
Mammals	$2 \times 10^{-9}$ / base / year
mtDNA nucleotides	
Mammals	5–10 × nuclear
Quantitative characters	
Fruit flies, mice and maize	$10^{-3} \times V_E^a$ / trait

<sup>a</sup>  $V_E$  is the environmental variance for the trait ([Chapter 5](#)).

*Sources:* Houle *et al.* (1996); Kumar & Subramanian (2002); Hedrick (2005a).

Mutation rates differ among classes of loci (Lynch *et al.* [2006](#)): microsatellite loci in mammals > loci causing morphological mutations > electrophoretic variants. Mutation rates per nucleotide base are clearly lower than rates per locus, as there are typically 1000 or more bases per locus.

Mutation rates for quantitative characters are approximately  $10^{-3}$  times the environmental variance per generation for a range of characters across a

range of eukaryotic species (Table 7.1). This apparently high rate, compared to single loci, arises because a mutation at any of the many loci underlying the character can affect the trait.

Spontaneous mutation rates are relatively constant over time. However, they may be elevated under stressful conditions, including those due to environmental agents (radiation, chemical mutagens and air pollution) and movement of mobile genetic elements (Hoffmann & Parsons 1997; Samet *et al.* 2004). As stresses have only a modest effect on mutation rate, they are unlikely to materially influence conclusions we reach about the evolutionary impact of mutation.

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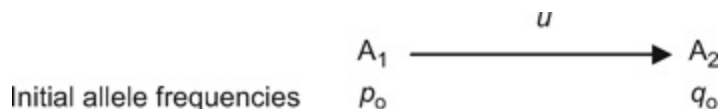
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As mutation rates are very low, mutation is a very weak evolutionary force and can often be ignored

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Mutation is normally a recurrent process where mutations continue to arise over time. We can model the impact of mutation on a population by considering a single locus with two alleles  $A_1$  and  $A_2$  at frequencies of  $p$  and  $q$ , with mutations only changing  $A_1$  into  $A_2$  at a rate of  $u$  per generation, as follows:



The frequency of the  $A_1$  allele in the next generation  $p_1$  is the frequency of alleles that do not mutate, namely

$$p_1 = p_0 (1 - u)$$

(7.1)

Thus, the frequency of the  $A_1$  allele declines.

The change in frequency of the  $A_1$  allele ( $\Delta p$ ) is the difference between the frequencies in the two generations

$$\Delta p = p_1 - p_0 = p_0(1 - u) - p_0$$

$$\Delta p = -up_0$$

$$\Delta p = -up_0$$

(7.2)

Consequently, the frequency of  $A_1$  declines by an amount that depends on the mutation rate  $u$  and the starting frequency  $p_0$ . There is a corresponding increase in the frequency of  $A_2$ . Since the mutation rate is approximately  $10^{-5}$  for morphological mutations, the maximum change in allele frequency per generation is  $10^{-5}$  when  $p = 1$ . This is very small and can be ignored in many circumstances, especially in the short term.

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Genetic diversity is only regenerated by mutation over periods of hundreds to millions of generations

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When genetic diversity is lost from a species, it is only regenerated extremely slowly by mutation. Regeneration times are very long, typically taking thousands to millions of generations for single-locus variation ([Box 7.1](#)). They depend on mutation rates, and are shorter for quantitative characters and

microsatellites (in mammals) and longer for allozymes and single-locus morphological variation ([Chapter 15](#)).

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### **Box 7.1 Time to regenerate genetic diversity by mutation**

Several endangered species, including the cheetah and the northern elephant seal, have lost much of their genetic diversity, presumably as a consequence of small population size (Bonnell & Selander [1974](#); O'Brien [1994](#); [Chapters 3, 8 and 11](#)).



Cheetah

The lost genetic diversity is only regenerated by mutation. Below we ask how long it would take to regenerate a frequency of 0.5 for an allele that had been lost. From [Equation 7.1](#), the change in allele frequency due to mutation is

$$p_1 = p_0 (1 - u)$$

This equation represents any single generation transition in frequency, so we can write

$$p_2 = p_1 (1 - u)$$

and by substituting for  $p_1$  from above, we obtain

$$p_2 = p_0 (1 - u)^2$$

Consequently the expression for the frequency after  $t$  generations  $p_t$  is:

$$p_t = p_0 (1 - u)^t \sim p_0 e^{-ut}$$

By taking natural logarithms ( $\ln$ ) and rearranging, we obtain the following expression for the number of generations ( $t$ ) for the frequency to change from  $p_0$  to  $p_t$  as

$$t = \frac{\ln p_0 - \ln p_t}{u}$$

The conditions we specified translate into  $p_0 = 1$  and  $p_t = 0.5$ . If we consider allozymes with a mutation rate of approximately  $u = 10^{-6}$ , then

$$t = \frac{(\ln 1 - \ln 0.5)}{10^{-6}} = 693147 \text{ generations}$$

For microsatellites in mammals  $u = 10^{-4}$ , so the number of generations will be

$$t = \frac{(\ln 1 - \ln 0.5)}{10^{-4}} = 6931 \text{ generations}$$

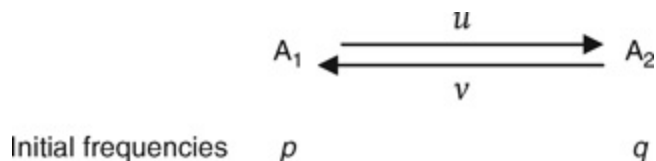
Clearly, genetic diversity is only regenerated very slowly by mutation. More detailed consideration of the regeneration of diversity suggests that  $10^5$ – $10^7$  generations are required to regenerate single-locus morphological or allozyme diversity and  $10^4$  generations to regenerate microsatellite genetic diversity in mammals (Lande & Barrowclough [1987](#)).



The balance between forward and reverse mutations results in an equilibrium where both alleles are present. Other, stronger forces often overwhelm this

If the mutation rates in the two directions are  $u$  and  $v$ , an equilibrium occurs when the mutations occurring in each direction are equal ( $up = vq$ ), resulting in an equilibrium frequency  $\hat{q}$ :

$$\hat{q} = \frac{u}{(u + v)} \quad (7.3)$$



Thus, the equilibrium frequency for the  $A_2$  allele depends only on  $u$  and  $v$ , the forward and reverse mutation rates. This is a **stable equilibrium** as the frequencies move back towards the equilibrium point, if perturbed.

To obtain the equilibrium frequency, we insert numerical values of forward and reverse mutation rates (Example 7.1). It takes many generations to reach this equilibrium, so we can only observe it in species with short generation times, e.g. bacteria. As the forces generating this equilibrium are very weak, it is easily overwhelmed by other stronger factors, such as natural selection.

**Example 7.1 Equilibrium frequency due to the balance between forward and reverse mutations**

Substituting typical values of  $u = 10^{-5}$  and  $v = 3 \times 10^{-6}$  into [Equation 7.3](#), we obtain:

$$\hat{q} = \frac{u}{(u + v)} = \frac{10^{-5}}{10^{-5} + (3 \times 10^{-6})} = \frac{10}{13} = 0.77$$

Thus, the equilibrium frequency of the  $A_2$  allele is 77% and that of the normal  $A_1$  allele 23%.

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## Mutation–selection balance and the mutation load

### Selective value of mutations

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The majority of newly arisen mutations are deleterious

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New mutations are being continually added to populations, albeit at a slow rate. These consist of:

- neutral mutations
- deleterious mutations (from mildly deleterious to lethal)
- occasional favourable mutations, and
- mutations favoured in some circumstances, but not in others.

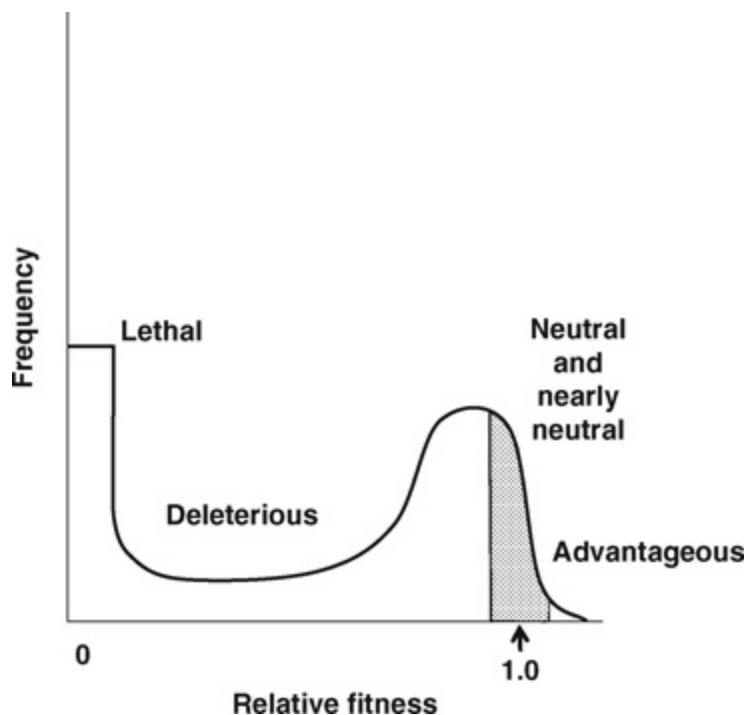
Most mutations outside functional loci are expected to be neutral or nearly so. Mutations within functional loci will be predominantly deleterious as random changes in the DNA sequence of a locus will usually be from a functional

allele to a less-functional state. A small proportion of mutations is advantageous (Bataillon 2003). There is limited evidence about the proportions of mutations that fall into the different categories, but those with phenotypic effects are predominantly deleterious, as mean fitness declines when spontaneous or induced mutations accumulate (Garcia-Dorado *et al.* 1999; Yang *et al.* 2001).

The distribution of the effects of mutations is a critical issue in two conservation contexts:

- maintenance of genetic diversity (Chapter 9)
- accumulation of newly arisen mildly deleterious mutations (Chapter 15).

Figure 7.1 illustrates our guesstimates on the proportions of mutations that fall into the different categories. About 90% of spontaneous mutations in fruit flies are deleterious and of these about 50% are lethal (Simmons & Crow 1977). Only 10% fall in the neutral and near-neutral categories (Lande 1995). The proportion of mutations that is favourable is presumed to be no more than 1–2%.



**Fig. 7.1** Hypothetical distribution of effects of new mutations in functional

loci on reproductive fitness. *Some mutations are lethal, most are deleterious, some are neutral, or near neutral (shaded region) and a small proportion are advantageous.*

Some mutations are deleterious in some conditions, but favourable in others and contribute to genotype  $\times$  environment interactions (Kondrashov & Houle 1994). However, we are unaware of any estimates of the proportion of mutations falling into this class. It is very difficult to study mutations, especially those of very small effect, as they require vast experiments to detect them.

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Deleterious mutations occur at thousands of loci in the genome, so their cumulative rate is relatively high

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The cumulative rate of recessive lethal mutations is about 0.01 per haploid genome per generation in fruit flies, nematodes and ferns (Drake *et al.* 1998). We can readily account for this in fruit flies as the product of the mutation rate per locus per generation ( $10^{-5}$ ) and the number of loci that can produce lethal mutations ( $\sim 3600$ : Miklos & Rubin 1996). As there are other deleterious mutations that are not lethal, the cumulative rate of deleterious mutation is considerably higher. This has been estimated to be 0.6 per haploid genome per generation in fruit flies, 0.1–0.8 in plants, 0.01 in rodents and 0.6–1.6 in humans, chimpanzees and gorillas (Drake *et al.* 1998; Eyre-Walker & Keightley 1999; Haag-Luitard *et al.* 2007). Other estimates are, if anything, higher than these (Lynch *et al.* 1999). While there are uncertainties about these estimates, the overall rates of deleterious mutation per genome are relatively high.

## The mutation load

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Low frequencies of deleterious alleles are found at many loci in all outbreeding populations, due to the balance between their origin by mutation and their removal by natural selection

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While selection removes deleterious alleles from populations, this often takes many generations, especially for recessive alleles. A **mutation–selection balance** (equilibrium) is reached between addition of deleterious alleles by mutation and their removal by selection. Consequently, low frequencies of deleterious alleles are found in all naturally outbreeding populations (**mutation load**), as illustrated for humans in [Table 7.2](#). These mutations are extremely important in understanding the deleterious consequences of inbreeding because inbreeding increases the probability that they are expressed in homozygous genotypes ([Chapters 12](#) and [13](#)).

**Table 7.2** *Frequencies of deleterious mutations in Caucasian humans, listed according to mode of inheritance*

Disease	Frequency
Autosomal dominant	
Achondroplasia	$5 \times 10^{-5}$
Retinoblastoma	$5 \times 10^{-5}$
Huntington's chorea	$5 \times 10^{-4}$
Autosomal recessive	
Albinism	$3 \times 10^{-3}$
Xeroderma pigmentosum	$2 \times 10^{-3}$
Phenylketonuria	$7 \times 10^{-3}$
Cystic fibrosis	$2.5 \times 10^{-3}$
Tay-Sachs syndrome	$1 \times 10^{-3}$
Sex-linked recessive	
Haemophilia	$1 \times 10^{-4}$
Duchenne's muscular dystrophy	$2 \times 10^{-4}$

Source: Nei (1975).

Several important points summarize mutation loads:

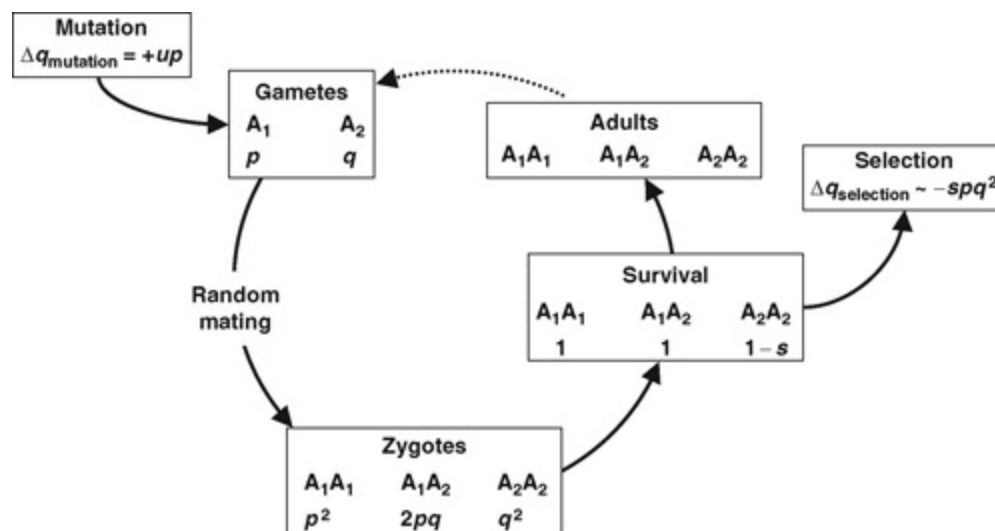
- mutational loads are found in all species, including threatened species (Table 7.3)
- deleterious alleles are normally found only at low frequencies, typically much less than 1% at any locus
- deleterious alleles are found at many loci
- there are characteristic differences in frequencies of deleterious alleles in populations according to the mode of inheritance and dominance (Table 7.2)
- most deleterious mutations are partially recessive.

**Table 7.3** *Deleterious mutations found segregating in threatened species*

Species	Deleterious mutation	Reference
Brown bear	albinism	1
California condor	chondrodystrophy	2
Florida panther	cryptorchidism, cardiac defects	3
Golden lion tamarin	hernia, liver enzyme defect	4, 5
Gray wolf	blindness	1
Maned wolf	cryptorchidism, cystinuria, epilepsy	1
Przewalski's horse	vitamin E malabsorption	4
Red ruffed lemur	hairlessness, funnel chest	4
Racoon	albinism	1
Tiger	partial albinism	1

References: 1, Laikre (1999); 2, Ralls *et al.* (2000); 3, Roelke *et al.* (1993); 4, Ryder (1988); 5, Schulman *et al.* (1993).

The mutation–selection equilibrium frequency for a given mode of inheritance depends only on the mutation rate and the selection coefficient. To understand the different allele frequencies for genetic diseases with different modes of inheritance, we derive expressions for allele frequency equilibria due to mutation–selection balance using the flowchart in Fig. 7.2.



**Fig. 7.2** Flowchart for mutation–selection balance.

For a deleterious recessive allele, the change in allele frequency due to the combined impacts of mutation ( $\Delta q_{\text{mutation}}$ ) and selection ( $\Delta q_{\text{selection}}$ ), is:

$$\Delta q = \Delta q_{\text{mutation}} + \Delta q_{\text{selection}}$$

Deleterious alleles increase due to mutation ( $up$ ) (Equation 7.2) and are removed by selection  $[-spq^2/(1 - sq^2)]$  (Table 6.2b). Therefore

$$\Delta q = up - \frac{spq^2}{1 - sq^2} \sim up - spq^2$$

(the denominator is essentially 1 as  $sq^2$  is a very small quantity for rare alleles)

At equilibrium,  $\Delta q = 0$ , so

$$up \sim spq^2$$

and

$$q^2 \sim \frac{u}{s}$$

Thus, the equilibrium frequency  $\hat{q}$  is:

$$\hat{q} \sim \sqrt{\frac{u}{s}} \quad (7.4)$$

The equilibrium between mutation and selection depends only upon the mutation rate and the selection coefficient. This equilibrium results in low frequencies of deleterious alleles in random mating populations. The frequency will be highest for mildly deleterious alleles, and least for lethal alleles. For example, if the mutation rate is  $10^{-5}$ /locus per generation, and the mutation is lethal ( $s = 1$ ), the equilibrium frequency is  $\sqrt{10^{-5}/1} = 3.2 \times 10^{-3}$ , while if the selection coefficient is 0.1, the equilibrium frequency is  $10^{-2}$ .



For mutations that are equally deleterious, autosomal recessives have the highest equilibrium frequencies and autosomal dominants the lowest, with sex-linked recessives intermediate

Equilibrium frequencies for autosomal and sex-linked loci with different degrees of dominance, derived using the same rationale as above, are shown in [Table 7.4](#). While the equilibria differ depending upon degree of dominance and whether the locus is autosomal or sex-linked, all depend only on the mutation rate and the selection coefficient. Equilibrium frequencies are higher for recessive than for dominant alleles, with additive alleles intermediate (for alleles with the same selection coefficients and mutation rates). Further, for recessives the equilibrium frequencies are higher for autosomal than for sex-linked loci due to the greater efficiency of selection against recessives in the hemizygous sex. Overall, the equilibrium frequencies accord with the observed patterns of frequencies of deleterious alleles in humans ([Table 7.2](#)).

**Table 7.4** *Mutation–selection equilibrium frequencies in diploids and tetraploids for alleles with different degrees of dominance. Examples of the expected equilibrium frequencies for lethal alleles ( $s = 1$ ) with mutation rates ( $u$ ) of  $10^{-5}$  are shown, along with that for a partially recessive lethal ( $h_s = 0.02$ )*

Mode of inheritance and dominance	Equilibrium frequency ( $\hat{q}$ )	Expected equilibrium frequency for lethals
Diploid		
Autosomal		
Recessive	$\sqrt{(u/s)}$	$3.16 \times 10^{-3}$
Partial recessive	$u/hs$	$5 \times 10^{-4}$
Dominant	$u/s$	$10^{-5}$
Additive	$2u/s$	$2 \times 10^{-5}$
Sex-linked		
Recessive	$3u/s$	$3 \times 10^{-5}$
Haploid	$u/s$	$10^{-5}$
Tetraploid		
Recessive		
allotetraploid	$(u/s)^{1/4}$	0.056
autotetraploid		
close to centromere	$(u/s)^{1/4}$	0.056
distant from centromere	$< (u/s)^{1/4}$	$< 0.056$
Partial recessive		
allotetraploid	$u/hs$	$5 \times 10^{-4}$

Sources: Lande & Schemske (1985); Falconer & Mackay (1996).

Alleles are rarely completely recessive (Simmons & Crow 1983). For example, ‘recessive’ lethals in fruit flies generally reduce heterozygote fitness by 1–3%. This has a substantial effect on their equilibrium frequencies, with a 2% reduction in heterozygote survival resulting in an equilibrium frequency for a lethal of  $5 \times 10^{-4}$ , nearly an order of magnitude lower than that for a completely recessive lethal (Table 7.4).

## Mutation–selection balance in polyploids

Mutation–selection equilibrium frequencies for recessives are higher in polyploids than in diploids, but similar for partial recessives

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As many species of plants are polyploid ([Chapters 16 and 17](#)), we need to consider mutation–selection equilibria in them as a prelude to considering the impact of inbreeding in polyploids versus diploids ([Chapters 12 and 13](#)). We will concentrate on tetraploids, as they are sufficient to illustrate the relevant principles.

Equilibrium frequencies for completely recessive alleles are typically higher in tetraploids than for equivalent diploid loci ([Table 7.4](#)). For example, a lethal with a mutation rate of  $10^{-5}$  will have an equilibrium frequency of about 0.056 in a tetraploid and  $3 \times 10^{-3}$  in a diploid. There is more opportunity for deleterious recessive alleles to be hidden from selection as ploidy rises, i.e. selection against deleterious alleles is most effective for haploid loci and declines progressively for sex-linked, diploid and tetraploid loci. Equilibrium frequencies under mutation–selection balance rise correspondingly. By contrast, partial recessive mutations have similar equilibria in diploids and tetraploids. As most deleterious mutations appear to be partial recessives, the latter situation is more realistic.

### **Estimating mutation rates from mutation–selection balance**

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Many estimates of mutation rates have been obtained from the balance between mutation and selection

---

It is extremely difficult to detect recessive mutations in diploid species as the genotypes of heterozygotes and homozygous normals cannot be distinguished phenotypically. For example, haemophilia, the sex-linked bleeding disease, is

frequent in the royal families of Europe, but it is not possible to determine in whom the mutation occurred, or to distinguish heterozygous females.

Estimates of mutation rates can be obtained using the equations for mutation–selection equilibrium. For example, the first estimate of a mutation rate in humans was obtained for sex-linked haemophilia, based on estimates of the equilibrium frequency and on the selection coefficient derived from hospital records (Example 7.2). The estimate of approximately  $3 \times 10^{-5}$  was in line with estimates from fruit flies and plants, species where mutations rates had been estimated by more direct methods.

---

### **Example 7.2 Estimating the mutation rate for haemophilia in humans from mutation–selection equilibrium (after Falconer & Mackay 1996)**

Haldane recognized that the equation for mutation–selection equilibrium could be rearranged to provide an estimate of the mutation rate. Taking the equation for mutation–selection equilibrium for a sex-linked recessive (Table 7.4) and rearranging, we obtain:

$$u = \frac{s\hat{q}}{3}$$

Using hospital records in Denmark, Haldane estimated that the frequency of haemophilia in males ( $q$ ) was approximately  $10.5 \times 10^{-5}$ . The survival rate of haemophiliacs relative to normal males ( $1 - s$ ) was 0.25. Thus, the selection coefficient is 0.75. Consequently, the estimate of mutation rate is

$$u = \frac{s\hat{q}}{3} = \frac{(0.75 \times 10.5 \times 10^{-5})}{3} = 2.6 \times 10^{-5}$$

Thus, the first estimate of mutation rate in humans was approximately  $3 \times 10^{-5}$ .

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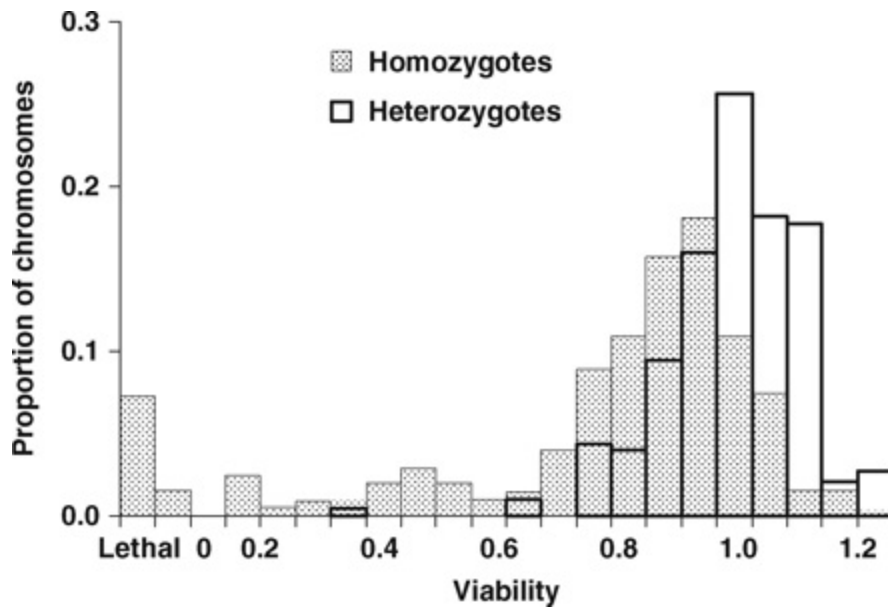
## Mutation–selection balance and fitness

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Outbreeding populations contain a load of rare partially recessive alleles that reduce reproductive fitness when homozygous

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Mutation–selection balance affects both single loci and quantitative characters, such as reproductive fitness. The impact of these deleterious alleles on fitness can be measured experimentally by making chromosomes homozygous. Most chromosomal homozygotes in fruit flies have reduced egg–adult survival (viability) ([Fig. 7.3](#)). The effects on total reproductive fitness are even greater, mean fitness being reduced by 70–90% compared to that of chromosomal heterozygotes in a range of tests (Latter & Sved [1994](#)). Almost all chromosomes sampled from outbreeding species carry alleles that are deleterious when homozygous, but different samples of a particular chromosome carry different complements of deleterious alleles, i.e. individually rare deleterious alleles are found at many separate loci. Inbreeding experiments lead to conclusions similar to those described above and extend them to a very wide range of species, including endangered species ([Chapter 13](#)).



**Fig. 7.3** Viabilities of second chromosome homozygotes and heterozygotes in fruit flies (after Hedrick 1983). Only 40% of the genome is being made homozygous in this experiment. *The average effect on fitness of making a chromosome homozygous is deleterious, with some homozygotes being lethal, a majority less deleterious, and a minority relatively normal.*

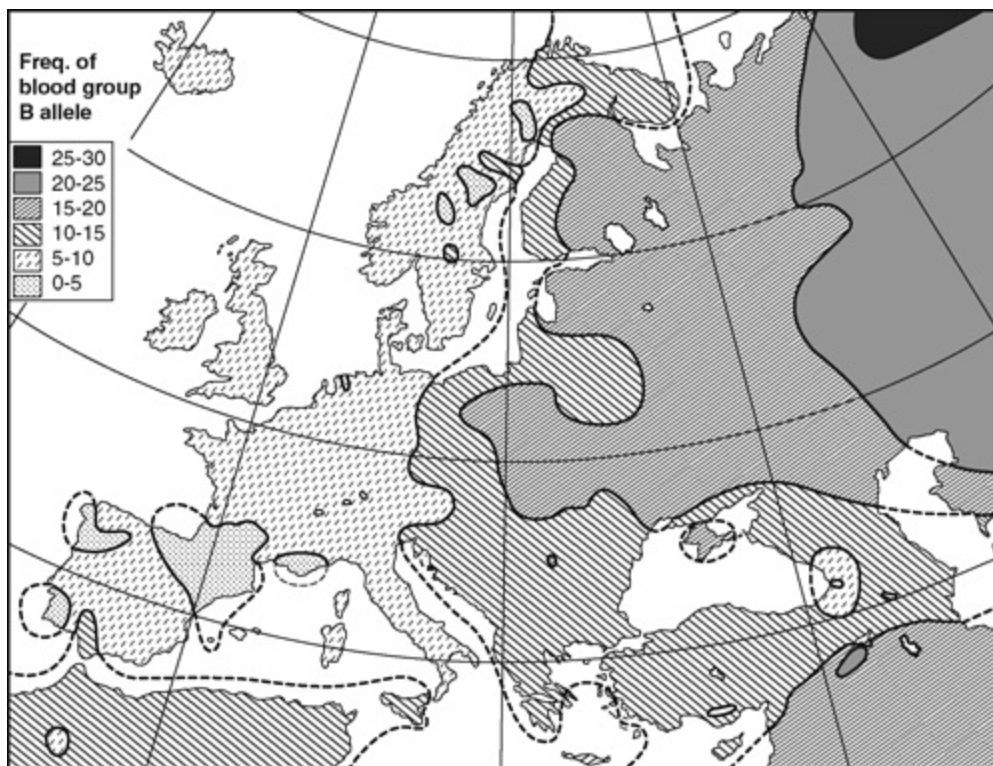
## Migration

The introduction of immigrants (gene flow) from one population into another reduces genetic differentiation among populations and usually restores lost genetic diversity

The gene pools of populations diverge over time as a result of chance and selection. Such differences are reduced by gene flow (Chapter 14). The impact of migration is illustrated by B blood group allele frequencies in human populations across Eurasia (Fig. 7.4). Prior to about AD 500, the B allele was essentially absent from Western Europe, but it existed in high

frequencies in the east. The Mongol and Tartar invasions of Europe between AD 500 and 1500 left a trail of rape and pillage and left some B alleles behind, generating a gradual decrease in frequency of the B blood group allele from east to west (termed a **cline**).

The genetic impact of migration depends on the proportion of alleles contributed by migrants and on the difference in frequency between the native population and the immigrants



**Fig. 7.4** B blood group allele frequencies across Eurasia, resulting from the Mongol and Tartar invasions between AD 500 and 1500 (Reprinted from *The Distribution of the Human Blood Groups and Other Polymorphisms*, Mourant, A.E., C. Kope and K. Domaniewska-Sobczak (1976) 2nd edn, by permission of Oxford University Press © Oxford University Press 1976.). It

is presumed that prior to this, the B blood group allele was absent from western Europeans, as it remains absent in native Basques in Spain, and in other isolated populations.

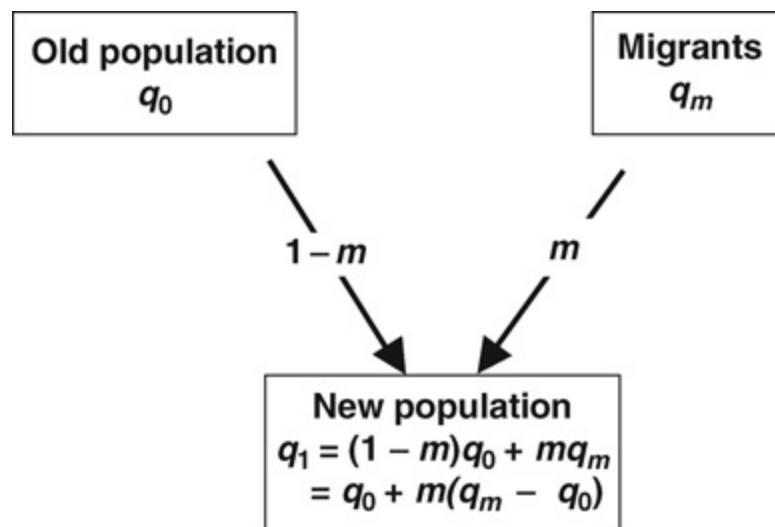
The change in allele frequency due to migration as modelled in [Box 7.2](#) is

$$\Delta q = m(q_m - q_0) \quad (7.5)$$

Thus, the change in allele frequency depends on the proportion of alleles contributed by migrants ( $m$ ), and on the difference in frequency between the immigrants ( $q_m$ ) and the native (original) population ( $q_0$ ). Migration may have very large effects on allele frequencies. For example, if immigrants are all homozygous for an allele absent from the native population, and 20% of the population in the next generation are immigrants, then the immigrant allele increases in frequency from 0 to 0.2 in a single generation.

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### **Box 7.2 Modelling the impact of migration on the genetic composition of a population**



The new population, after migration, is made up of a proportion of



residents  $(1 - m)$  with a frequency  $q_0$  and a proportion of migrants  $(m)$  with their frequency  $q_m$ . Therefore,

$$q_1 = (1 - m)q_0 + mq_m = q_0 + m(q_m - q_0)$$

Change in frequency from the original population ( $q_0$ ) to the new population after immigration ( $q_1$ ) is:

$$\begin{aligned}\Delta q &= q_1 - q_0 = q_0 + m(q_m - q_0) - q_0 \\ &= m(q_m - q_0)\end{aligned}$$

Equation 7.5 can be used to estimate the migration rate from allele frequency data, as illustrated for the extent of admixture from domestic dog genes in the endangered Ethiopian wolf in Example 7.3. Many species are threatened by gene flow (**introgression**) from related non-endangered species (Chapter 18).

### Example 7.3 Estimating dog introgression in the endangered Ethiopian wolf from microsatellite allele frequencies (data from Gottelli et al. 1994)

Ethiopian wolves are genetically distinct from domestic dogs, but hybridization occurs in areas where they co-occur, as in Web Valley, Ethiopia (Box 4.2). The population from the Sanetti Plateau is relatively pure. Dogs lack the *J* allele at microsatellite locus 344, while ‘pure’ Ethiopian wolves are homozygous for it. Frequencies of this allele are:

		<i>J</i> allele frequency
Sanetti population	$q_0$	1.00 (‘old’)
Web population	$q_1$	0.78 (‘new’ – containing dog admixture)
Domestic dogs	$q_m$	0.00 (‘migrants’)

All the non-*J* alleles in the Web population have come from dogs. The extent of admixture from domestic dogs in the Web population of Ethiopian wolves can be estimated using the equation from [Box 7.2](#). This can be rearranged to provide an expression for the migration rate *m*, as follows:

$$q_1 = q_0 + m(q_m - q_0)$$
$$m = \frac{q_1 - q_0}{q_m - q_0}$$

Upon substituting allele frequencies from above into this expression, we obtain

$$m = \frac{0.78 - 1.0}{0 - 1.0} = 0.22$$

Thus, the Web Valley population of Ethiopian wolves contains about 22% of its genetic composition from domestic dogs. This is the accumulated contribution of alleles from dogs, not a per generation estimate. Phenotypically abnormal individuals, suspected of being hybrid individuals, represent about 17% of the population. Estimates can also be made from other microsatellite loci and the best estimate would come from combining data from all informative loci.

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## Migration–selection equilibria and clines

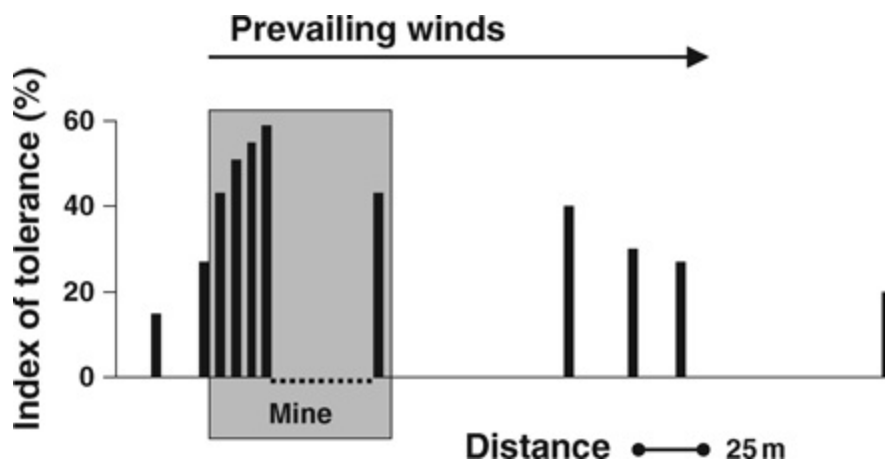
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Migration among populations subject to differential selection may lead to gradation in allele frequencies (clines)

Clines can form where there is a balance between selection favouring different alleles in diverse habitats (local adaptation) and migration occurring between the habitats (gene flow). For example, there is a cline in heavy-metal tolerance in colonial bent grass plants passing from polluted mine wastes to nearby unpolluted pasture in Wales. Selection favours heavy-metal tolerant plants on the mine waste, but acts against them in the unpolluted surrounding pasture, and pollen flow moves alleles among the populations ([Box 7.3](#)).

### **Box 7.3 Cline in heavy-metal tolerance in colonial bent grass due to migration–selection equilibrium (Bradshaw & McNeilly 1981)**

Genetically determined heavy-metal tolerance is high on the polluted slagheaps from old mines in Wales, but low in the surrounding pastures that are relatively unpolluted by heavy metals. Pollen flows predominantly from plants on mine wastes in the direction of the prevailing wind, creating a gradient of heavy-metal tolerance that declines with distance from the mine. Selection favours heavy-metal tolerance on the mine wastes, and acts against tolerant plants on the normal pasture. Heavy-metal tolerance has evolved in several different species of grasses (including colonial bent grass, *Anthoxanthum odoratum* and *Festuca ovina*), allowing them to colonize polluted mine tailings (Jones & Wilkins 1971; Briggs & Walters 1997).



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Clines are common for morphological and quantitative characters. In fact, they are so common that they are accorded the status of ecogeographic rules (Futuyma [1998](#)). Bergmann's rule states that races from cooler climates are larger than races from warmer climates. Allen's rule states that in warm-blooded animals, protruding parts (e.g. ears, tail) are shorter in races from colder climates than in races from warmer climates. Gloger's rule states that races of warm-blooded vertebrates are more darkly pigmented in warm and humid areas than in cool and dark areas.

While these rules refer to phenotypes, they typically reflect both environmental and genetic differences. Fruit flies in Europe show a latitudinal cline in wing length. This is due, at least partially, to genetic differences, as it is found when flies from different regions are raised under the same laboratory conditions. This cline is due to natural selection as it evolved again, within 20 years, when fruit flies were introduced into North America (Huey *et al.* [2000](#)). Clines seem to be more frequent in quantitative characters than for allozymes or DNA markers (see Hedrick & Savolainen [1996](#)).

Migration and selection may also act in the same direction and speed the spread of an allele. For example, a single allele in fruit flies that confers resistance to DDT and a range of other insecticides has spread globally since the 1930s, in spite of quarantine barriers (Daborn *et al.* [2002](#)).

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The shape of clines is related to species' dispersal ability

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Clines are expected to be steep (i.e. large changes over short distances) when dispersal rates are low and more gradual when dispersal rates are high. For example, clines in the frequency of industrial melanic versus typical moths from highly polluted to essentially unpolluted regions of Britain are steeper for the weakly dispersing scalloped hazel moth than for the more strongly dispersing peppered moth (Bishop & Cook 1975). Species that disperse readily over long distances show less local differentiation and fewer clines than species with lesser dispersal ability (Chapter 14). Plants show considerable local adaptations to soil and climate (as they cannot escape them), and probably develop clines more frequently than animals.

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The migration–selection equilibrium frequency depends on the intensity of selection, the proportion of immigrants and on the allele frequency in the immigrants

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The simple model for the equilibrium between migration and selection in Fig. 7.5 shows that equilibrium depends only upon the migration rate ( $m$ ), the selection coefficient ( $s$ ), and the allele frequency in the migrants ( $q_m$ ), i.e. it does not depend on the allele frequency in the initial population. When migration rates are high and selection is weak, migration dominates the process and essentially erases local adaptation (‘swamping’). Conversely, when migration rates are low and selection is strong, there will be local adaptation. This is consistent with the effects of dispersal rates on the steepness of clines, as described above. Example 7.4 illustrates the equilibrium achieved for given values of the selection coefficient, migration rate and frequency in the immigrants.

$$\Delta q_{\text{selection}} = \frac{-\frac{1}{2}sq(1-q)}{1-sq} \sim \frac{1}{2}sq^2 - \frac{1}{2}sq \qquad \Delta q_{\text{migration}} = m(q_m - q)$$

$$\text{Overall } \Delta q = \Delta q_{\text{selection}} + \Delta q_{\text{migration}}$$

$$= \frac{1}{2}sq^2 - \frac{1}{2}sq + m(q_m - q) = \frac{1}{2}sq^2 - q(m + \frac{1}{2}s) + mq_m$$

(the denominator  $\sim 1$ , assuming  $sq$  is small).

At equilibrium  $\Delta q = 0$ , so the equilibrium frequencies are obtained as the solutions of the quadratic equation, yielding (Li 1976):

$$\hat{q} = \frac{(2m + s) \pm \sqrt{[(2m + s)^2 - 8smq_m]}}{2s} \quad (7.6)$$

While there are two solutions to the equation, only one of them will be possible (frequency between 0 and 1).

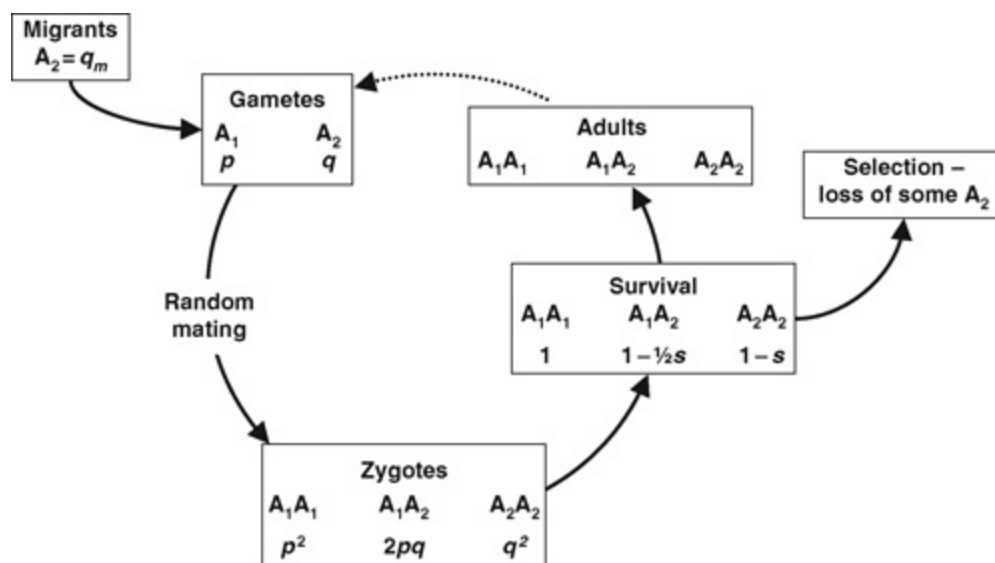
### Example 7.4 Migration–selection equilibrium

If  $s = 0.2$ ,  $m = 0.1$ , and  $q_m = 0.1$ , the equilibrium frequency is obtained by substituting these values into the expression for allele frequency equilibrium from [equation 7.6](#) above:

$$\begin{aligned} \hat{q} &= \frac{(2m + s) \pm \sqrt{[(2m + s)^2 - 8msq_m]}}{2s} \\ \hat{q} &= \frac{[(2 \times 0.1) + 0.2] \pm \sqrt{[(2 \times 0.1) + 0.2]^2 - (8 \times 0.1 \times 0.2 \times 0.1)}}{2 \times 0.2} \\ &= 0.0513 \text{ (or 1.95, an impossible solution)} \end{aligned}$$

$$= 0.0513 \text{ (or 1.95, an impossible solution)}$$

Thus, the equilibrium frequency is 5.1% due to the balance between migration and selection.



**Fig. 7.5** Model of the balance between migration and selection. A large random mating population is subject to additive viability selection that is being balanced by migration from a population with a different allele frequency.

Clines may reflect changes in selection across environmental gradients, such as temperature, altitude, etc. As such clines depend on the selective forces, they should change if the selective forces change. Movements of clines in allozyme loci and chromosomes in fruit flies in Australia, Europe and North and South America have been observed as a consequence of global climate change (Umina *et al.* 2005; Balanyá *et al.* 2006). Clines may also occur as the result of short-term historical migration events, as we saw previously (Fig. 7.4).

Clines due to migration–selection balance will be disrupted in threatened species if habitat fragmentation eliminates gene flow. This will alter the balance between local adaptation and migrational ‘swamping’ in the direction of local adaptation.

Migration–selection balance can arise between wild and captive populations of endangered species when there is regular movement of wild

individuals into captivity, or vice versa. For example, the wild population of nene (Hawaiian goose) is regularly augmented by captive-bred individuals, as are many fish species. Captive populations adapt to their captive environment and typically have reduced reproductive fitness when returned to their natural habitat ([Chapter 20](#)).

## Summary

1. Mutation is the ultimate source of all genetic diversity.
2. Mutation and migration are the only means for regaining genetic diversity lost through chance or selection.
3. Mutations occur at a very low rate, thus mutation is a very weak evolutionary force over the short term.
4. All naturally outbreeding populations contain a load of deleterious mutations due to mutation–selection balance. When populations are inbred, these mutations cause reduced reproductive fitness (inbreeding depression).
5. Migration (gene flow) reduces genetic differentiation caused by natural selection and chance.
6. Migration from introduced species compromises the genetic integrity of many endangered species.
7. Migration and selection may result in a balance, or a cline.

## Further reading

Falconer & Mackay ([1996](#)) *Introduction to Quantitative Genetics*. Provides a very clear treatment of the topics in this chapter.

Hartl & Clark (2007) *Principles of Population Genetics*. Covers many of the topics in this chapter.

Hedrick ([2005a](#)) *Genetics of Populations*. Has a more extensive treatment of many of the topics in this chapter.

## Software



EASYPOP: Free simulation software for population genetics allowing investigation of the effects of mutation, population size, migration and population fragmentation (Balloux 2001).  
[www.unil.ch/dee/page36926\\_fr.html](http://www.unil.ch/dee/page36926_fr.html)

WINPOP 2.5: Free software to simulate selection, drift, gene flow and migration. [www.genedrift.org/winpop.php/](http://www.genedrift.org/winpop.php/)

## Problems

- 7.1 Mutation. By how much will the frequency of a microsatellite allele in cheetahs change due to mutation in one generation, if the allele has a current frequency of 0.1 and the mutation rate from other alleles to this allele is  $10^{-4}$ ?
- 7.2 Forward and reverse mutation. What is the equilibrium frequency due to forward and reverse mutation at a locus where the mutation rates are  $10^{-5}$  and  $10^{-6}$ , respectively?
- 7.3 Regeneration of genetic diversity by mutation. Northern elephant seals lost all of their allozyme variation as a result of a population size bottleneck caused by over-exploitation. How many generations would it take for an allele that had been lost to reach a frequency of 0.4 due to mutation alone, assuming a mutation rate of  $4 \times 10^{-6}$ ?
- 7.4 Mutation–selection equilibrium. What equilibrium frequency would you expect for the recessive lethal chondrodystrophy in California condors as a result of mutation–selection balance, if the mutation rate was  $2 \times 10^{-5}$ ?
- 7.5 Mutation–selection equilibrium. What mutation rate would be needed to explain the current frequency of chondrodystrophy among captive hatchlings of about 17%? Is this a realistic mutation rate? Why do you think the frequency of the lethal allele is so high?
- 7.6 Mutation–selection equilibrium. Derive the equilibrium frequency for an additive autosomal locus as a result of mutation–selection equilibrium. Use the expressions from [Equation 7.2](#) and [Table 6.2](#) to obtain an expression for the overall change in allele frequency

(remember to insert  $\Delta q$ , not  $\Delta p$ ).

$$\Delta q = \Delta q_{\text{mutation}} + \Delta q_{\text{selection}} =$$

(Hint: it is reasonable to assume that the denominator of the selection term is approximately unity for a rare allele)

$$\text{At equilibrium } \Delta q =$$

$\therefore$

- 7.7** Mutation–selection equilibrium. Estimate the mutation rate for a dominant autosomal dwarfism allele in humans using the appropriate mutation–selection equilibrium equation. The phenotypic frequency of dwarfs at birth is 10/94 000, and their relative fitness is approximately 20% (after Strickberger 1985).
- 7.8** Mutation–selection equilibrium. Compute the equilibrium frequencies for loci that are autosomal recessive, additive autosomal, autosomal dominant, sex-linked recessive and haploid, given that the selection coefficient is 0.10 and the mutation rate  $10^{-5}$  per locus per generation. What are the impacts of ploidy (haploid vs. diploid) and dominance on the equilibrium frequencies?
- 7.9** Migration. Use the migration equation to estimate the extent of racial admixture in US African Americans in Georgia from  $Fy^a$  allele frequencies at the Duffy blood group locus (data from Strickberger 1985).

	$Fy^a$ frequency
Africans	0.000
US Caucasians	0.422
US African Americans	0.045

## Chapter 8 Genetic consequences of small population sizes

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Populations of conservation concern are small and/or declining in numbers. Small, isolated populations suffer accelerated inbreeding and loss of genetic diversity leading to reduced reproductive fitness (inbreeding depression) and reduced ability to evolve in response to environmental change

### Terms

Binomial distribution, bottleneck, effective population size, evolutionary potential, fixation, idealized population, inbreeding, Poisson distribution, random genetic drift, stochastic

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Mauritius kestrel: a species that survived a population size bottleneck of a single pair, but one that is genetically compromised

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## **Importance of small populations in conservation biology**

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Species of conservation concern have, by definition, small or declining population sizes

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Small or declining populations of threatened species are more prone to extinction than large stable populations. Species whose adult population sizes are less than 50, 250 or 1000 are designated as critically endangered, endangered and vulnerable, respectively, by IUCN ([Chapter 1](#)). Only ~100 critically endangered northern hairy-nosed wombats survive in Australia (adults plus juveniles), while the Mauna Kea silversword in Hawaii declined to about two dozen plants. Some species have reached such low numbers that they exist, or have existed, only in captivity. These include Arabian oryx, black-footed ferret, European bison, Père David's deer, Przewalski's horse, scimitar-horned oryx, California condor, Guam rail, 11 species of *Partula* snail, Cook's kok'io plant, Franklin tree and Malheur wirelettuce (WCMC [1992](#); Falk *et al.* [1996](#)).



Guam rail

Some species have experienced population size reductions (**bottlenecks**), but have since recovered. The Mauritius kestrel was reduced to a single pair but has now recovered to 400–500 birds ([Box 8.1](#)). Northern elephant seals were reduced to 20–30 individuals but now number over 175 000 ([Box 8.2](#)). These populations pay a genetic cost for their bottlenecks, typically having higher levels of inbreeding, reduced genetic diversity, lower reproductive fitness and compromised ability to evolve ([Box 8.1](#)).

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**Box 8.1 A population size bottleneck in the Mauritius**

## kestrel and its genetic consequences (after Groombridge et al. 2000)

The decline of the Mauritius kestrel began with the destruction of native forest and the plunge towards extinction resulted from thinning of egg shells and greatly reduced hatchability following the use of DDT insecticide which began in the 1940s. In 1974, its population numbered only four individuals, with the subsequent population descending from only a single breeding pair. Under intensive management the population grew to 400–500 birds by 1997.

However, the restored Mauritius kestrel carries genetic scars from its near extinction, including very low genetic diversity for microsatellite loci, compared to six other non-endangered kestrel populations. Prior to their decline, ancestral Mauritius kestrels had substantial genetic diversity, based on museum skins from 1829–1894, although this was also lower than that of the non-endangered species. The Seychelles kestrel went through a parallel decline and recovery and also has low genetic diversity.

Species	A	$H_e$
<b>Endangered</b>		
Mauritius kestrel		
Restored	1.41	0.10
Ancestral	3.10	0.23
Seychelles kestrel	1.25	0.12
<b>Non-endangered</b>		
European kestrel	5.50	0.68
Canary Island kestrel	4.41	0.64
South African rock kestrel	5.00	0.63
Greater kestrel	4.50	0.59
Lesser kestrel	5.41	0.70

The reproductive fitness of the Mauritius kestrel has been adversely affected by inbreeding in the early post-bottleneck population, with lower fertility and offspring productivity than in comparable falcons, and higher adult mortality in captivity.

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Small population size is a pervasive concern in conservation biology. Not only do such populations suffer the genetic costs described above and have an increased probability of extinction, but evolutionary processes in small populations differ from those in large populations. Consequently, it is critical that we understand the special evolutionary problems confronted by small populations.

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The role of chance is much greater, and the impact of selection is less, in small than large populations

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In large populations, mutation, selection and migration have essentially deterministic effects, and the effects of chance are generally minimal, except for neutral alleles. Conversely, in small populations, the role of chance predominates and the effects of selection are typically reduced or even eliminated. Chance introduces a random, or **stochastic**, element into the evolution of small populations, i.e. replicate loci and populations exhibit a diversity of outcomes. Small populations become inbred at a faster rate than do large populations, as inbreeding is unavoidable.

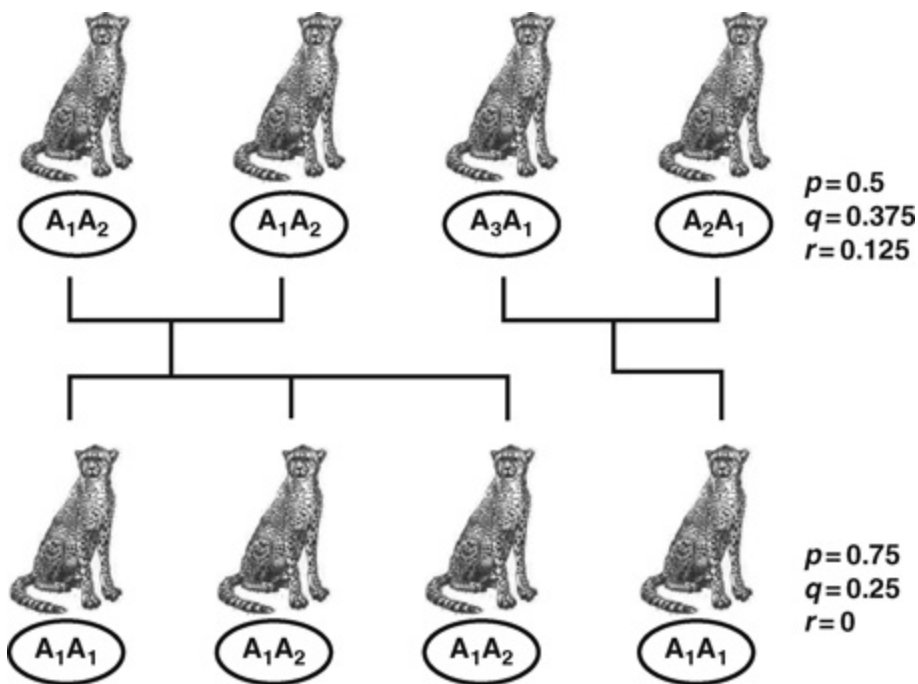
We begin by considering the effects of chance alone in small populations, followed in turn by inbreeding, selection, and mutation–selection equilibrium. The role of migration in small populations is deferred to [Chapter 14](#) where we consider population fragmentation.

## Chance effects

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Chance effects arise from random sampling of gametes in small populations

When a sexual population reproduces, the subsequent generation is derived from a sample of parental gametes. In small populations, some alleles (especially rare ones) may be lost just by chance and the frequencies of alleles that are transmitted to the following generation are likely to differ from those in the parents (Fig. 8.1). Allele frequencies fluctuate, or drift, from one generation to the next, a process termed random **genetic drift**.



**Fig. 8.1** Genetic drift in allele frequencies in a small population of cheetahs.  $p$ ,  $q$  and  $r$  are the frequencies of alleles  $A_1$ ,  $A_2$  and  $A_3$ , respectively. Allele  $A_3$  is lost by chance. Further, the frequencies of  $A_1$  and  $A_2$  change from one generation to the next, with  $A_1$  rising and  $A_2$  falling.



## Genetic drift

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Genetic drift has major impacts on the evolution of small populations

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It might seem that chance effects would have only minor impacts on the genetic composition of populations. However, random sampling of gametes within small populations has three consequences of major importance in evolution and conservation:

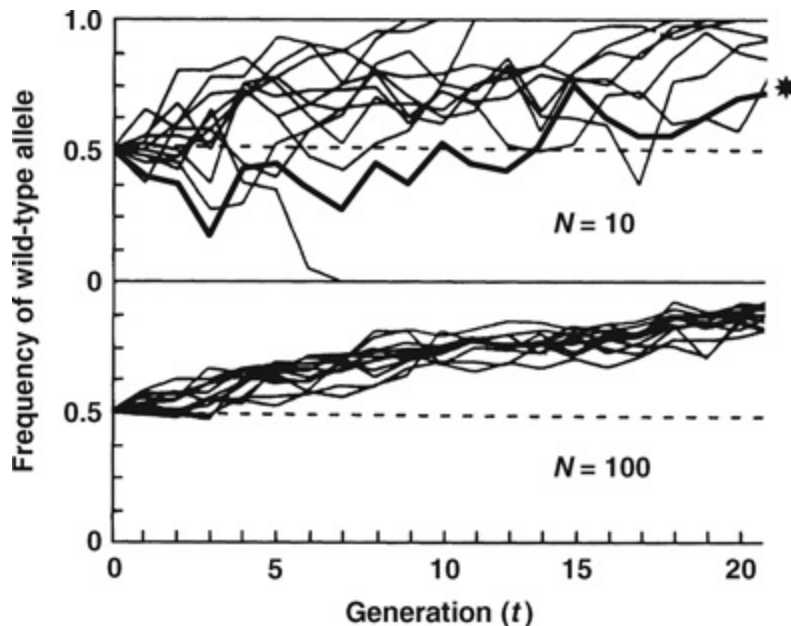
- loss of genetic diversity and fixation of alleles within populations, with consequent reduction in the ability to evolve
  - diversification of allele frequencies among replicate populations from the same original source (e.g. fragmented populations)
  - genetic drift overpowering natural selection.
- 

The effects of chance are greater in small than in larger populations

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These features are exemplified in the flour beetle populations in [Fig. 8.2](#). First, individual populations show genetic drift in allele frequencies over generations. For example, the allele marked with an asterisk in the upper panel begins at a frequency of 0.5, drops in frequency for three generations, and then rises and falls until generation 20 when its value is approximately

0.65. The fluctuations in frequencies for populations of size  $N = 10$  are much greater than for those with  $N = 100$ .



**Fig. 8.2** Random genetic drift of the wild-type (+) allele at a body colour locus in the red flour beetle. All 24 populations began with frequencies of 0.5 for the + and black alleles and were maintained by random sampling of either 10 ( $N = 10$ ), or 100 ( $N = 100$ ) individuals to be parents of each succeeding generation (after Falconer & Mackay 1996). *Much larger variation in allele frequencies occurred in the small than in the larger populations due to random genetic drift, both among replicates and from generation to generation in individual replicates. Selection favoured the wild-type allele.*

Second, there is random diversification among replicate populations, particularly in the  $N = 10$  populations. While all populations began with frequencies of 0.5, they ended up with frequencies ranging from 0 to 1. Again, the diversification among replicate populations is much less for the  $N = 100$  populations.

Third, some populations lose all genetic diversity and reach **fixation**. Over 20 generations in the  $N = 10$  populations, seven of the 12 became fixed, six for the wild-type allele and one for the black allele. None of the 12 large populations became fixed over the 20 generations.

Finally, selection favoured the wild-type allele over the black mutation. Nonetheless, genetic drift has overpowered this selection and resulted in fixation of the black allele in one  $N = 10$  population.

Naturally fragmented populations will experience these effects at all their genetic loci, with smaller fragments experiencing greater genetic drift than larger fragments.

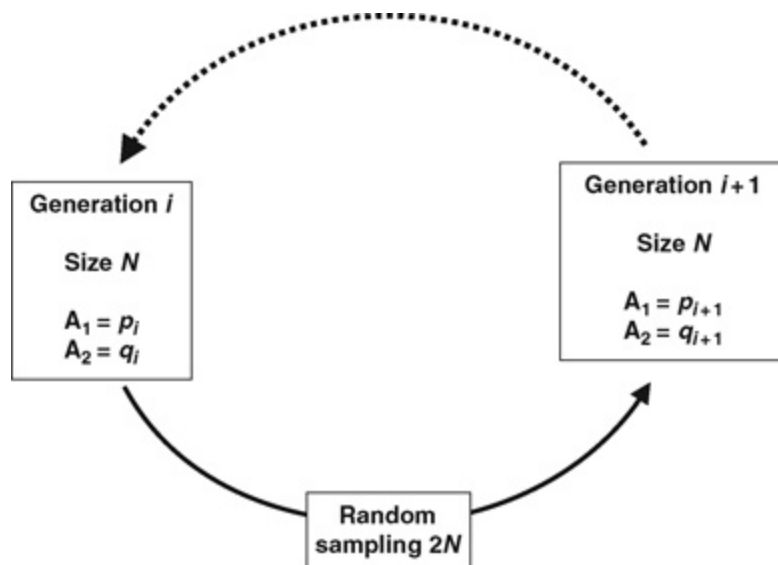
## Modelling drift in allele frequencies

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Genetic drift can be predicted using binomial sampling theory

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The characteristics of chance effects can be understood by modelling the sampling process in the absence of selection, mutation and migration ([Fig. 8.3](#)). We first consider sampling alleles for an offspring from a selfing diploid individual with genotype  $A_1A_2$ . This is akin to tossing two coins. The possible outcomes are two heads, two tails, a head and a tail, or a tail and a head, all with probabilities of  $\frac{1}{4}$ . The chance that only heads are obtained is  $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$ . Thus, if only one offspring is produced, there is a 50% chance that one or other allele will be lost. With  $N$  offspring, there are  $2N$  coins tossed and the chance that all are heads (or tails) is now  $(\frac{1}{2})^{2N}$ . Consequently, it is less likely that either  $A_1$  or  $A_2$  will be lost if  $N$  is large. Further, the allele frequencies in the offspring will be more similar to that of the parent in larger populations. As in statistical theory, a larger (genetic) sample size always provides a better estimate (of parental allele frequencies) than a smaller one.



**Fig. 8.3** Simple model of a small population with no mutation, migration or selection.

Variation in allele frequencies among replicate populations or loci depends on the allele frequencies and on the population size

When a population of size  $N$  reproduces,  $2N$  gametes are sampled to produce subsequent generations (Fig. 8.3). If the population being sampled has two alleles,  $A_1$  and  $A_2$ , at initial frequencies of  $p_0$  and  $q_0$ , respectively (and there are no other forces), the mean frequency of the  $A_1$  allele in the next generation ( $p_1$ ) over a large number of replicate populations is unchanged:

$$p_1 = p_0$$

However, there will be random variation among replicate populations in their allele frequencies. In the simple case where  $N = 2$  (Example 8.1), the

expected distribution of allele frequencies in replicate populations are given by terms of the binomial expansion of  $(p + q)^4$ . There are five possible outcomes, the first and the last outcomes being homozygous (fixed). Consequently, the average heterozygosity across all replicate populations is reduced (see below).

### Example 8.1 Expected distribution of allele frequencies in populations of size $N = 2$

If we take many samples of 2 individuals (= 4 gametes) from the same population where alleles  $A_1$  and  $A_2$  have frequencies of  $p$  and  $q$ , respectively, the expected distribution of allele frequencies is given by the terms of the binomial expansion  $(p + q)^4$ . The power of 4 is the number of gametes sampled. The terms of this expansion are given below, along with the frequencies for an example where  $p = 0.6$  and  $q = 0.4$ .

Outcome	$p$	frequency ( $f$ )	Example ( $p = 0.6, q = 0.4$ )
4 $A_1$ , 0 $A_2$	1.00	$p^4$	$0.6^4 = 0.1296$
3 $A_1$ , 1 $A_2$	0.75	$4p^3q$	$4 \times 0.6^3 \times 0.4 = 0.3456$
2 $A_1$ , 2 $A_2$	0.50	$6p^2q^2$	$6 \times 0.6^2 \times 0.4^2 = 0.3456$
1 $A_1$ , 3 $A_2$	0.25	$4pq^3$	$4 \times 0.6 \times 0.4^3 = 0.1536$
0 $A_1$ , 4 $A_2$	0.00	$q^4$	$0.4^4 = 0.0256$
Totals		1.0	1.0000

Thus, we expect five possible outcomes with frequencies of  $A_1$  of 1, 0.75, 0.5, 0.25 and 0. For the example with initial base population frequencies of  $p = 0.6$  and  $q = 0.4$ , the proportions of each outcome are expected to be 12.96%, 34.56%, 34.56%, 15.36% and 2.56%, respectively.

The mean frequency is

$$\begin{aligned}
\text{Mean } p &= \sum p_i f_i \\
&= [(1 \times 0.1296) + (0.75 \times 0.3456) + (0.5 \times 0.3456) \\
&\quad + (0.25 \times 0.1536) + 0] \\
&= 0.6
\end{aligned}$$

Thus, the mean frequency is unchanged.

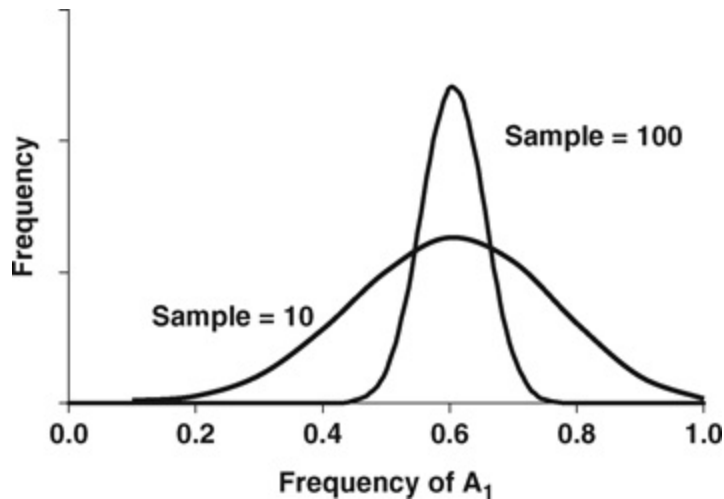
For a population with size  $N$ , the expected distribution of outcomes for a locus with two alleles is given by the terms of the binomial expansion  $(p + q)^{2N}$  (akin to the tossing of a biased coin). The probability that a population has all  $A_1$  alleles is  $p^{2N}$ , while the probability that it has all  $A_2$  alleles is  $q^{2N}$ . These two situations correspond to populations that have lost all their genetic diversity. The probability that a population has  $r$   $A_1$  alleles and  $(2N - r)$   $A_2$  alleles is

$$\binom{2N}{r} p^r q^{2N-r}$$

where  $\binom{2N}{r}$  is the binomial function  $2N! / [r! (2N - r)!]$ .

The expected distributions of allele frequencies over many replicate populations of sizes 10 and 100 are shown in [Fig. 8.4](#). Among replicate populations, the variance around the mean allele frequency is given by the binomial sampling variance:

$$\sigma_p^2 = \frac{p_0 q_0}{2N} \quad (8.1)$$



**Fig. 8.4** Expected distribution of allele frequencies when many replicate samples of sizes 10 and 100 are taken from a population with an allele frequency for A<sub>1</sub> of 0.6.

Thus, the variance in allele frequency depends on the allele frequencies and the population size. Variances are higher in smaller than larger populations, and greatest when the two alleles have frequencies of 0.5. When there are two alleles,  $\sigma_p^2 = \sigma_q^2$ .

Sampling occurs in every generation in small populations, and the effects are cumulative. This can be seen in [Fig. 8.2](#), where the replicate populations of size 10 diverge more with passing generations. The cumulative effects of genetic drift over generations are extremely important in conservation genetics, but are deferred until [Chapters 11–14](#).

## Fixation

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The probability of losing an allele in a single generation is higher in a small than in a large population and greater for rare than for common alleles

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Genetic drift will ultimately cause all except one allele to be lost. The probability that a gamete does not contain allele  $A_1$  is  $(1 - p)$ . Consequently, the probability that a random mating population loses allele  $A_1$  (all individuals in the population become  $A_2A_2$ ) in a single generation, is the probability that a gamete does not contain allele  $A_1$  raised to the power of the number of gametes sampled, namely

$$\text{Pr(losing } A_1) = (1 - p)^{2N}$$

Similarly, the chance of losing allele  $A_2$  (all individuals becoming  $A_1A_1$ ), is  $(1 - q)^{2N}$ . For example, the chance of losing  $A_1$  from one generation of sampling for a diploid population of size four, with alleles  $A_1$  and  $A_2$  at initial frequencies of 0.25 and 0.75, respectively, is  $(1 - 0.25)^8 = 0.100$ . The probability of losing  $A_2$  is  $(1 - 0.75)^8 = 1.53 \times 10^{-5}$ . Note that the rarer allele has a far greater probability of being lost. For a population of size 100, the chance of losing alleles with these frequencies in one generation is essentially zero.

## Effects of population bottlenecks

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Genetic diversity is reduced by population bottlenecks

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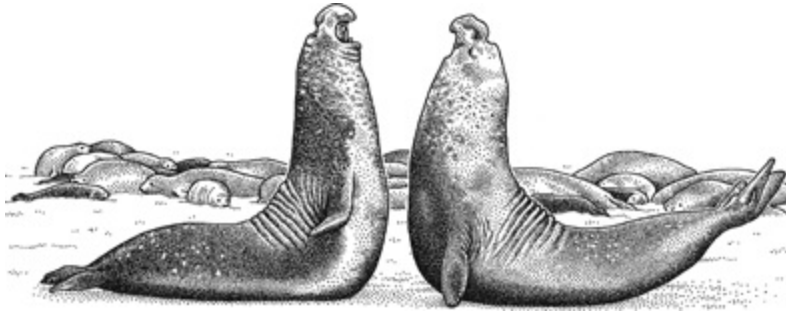
A bottleneck is a sharp reduction in population size; it may be short or long term. Many endangered species have been bottlenecked ([Table 8.1](#)) and on



average have reduced genetic diversity ([Chapter 3](#)). For example, the Mauritius kestrel and the northern elephant seal (previously endangered) have suffered bottlenecks and have reduced genetic diversity ([Boxes 8.1](#) and [8.2](#)).

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### **Box 8.2 Population bottleneck in the northern elephant seal ([Hoelzel 1999](#))**



Northern elephant seals suffered such a severe decline from hunting for fur and oil that they were thought to be extinct in the late nineteenth century. Fortunately, a small population of about 20–30 survived on Isla Guadalupe in the Pacific.

In their classic study, Bonnell & Selander ([1974](#)) showed that this bottlenecked population had no genetic diversity at 20 allozyme loci, while the related southern elephant seal had normal levels of genetic diversity. Subsequently, Hoelzel ([1999](#)) found that the northern elephant seal had only two mtDNA haplotypes, compared to 24 in southern elephant seals, and four in a sample of only five pre-bottleneck northern elephant seals (Weber *et al.* [2000](#)). The northern elephant seal also has reduced genetic variation compared to that in the southern elephant seal for DNA fingerprints, an immune system locus (MHC) and microsatellites (heterozygosities of 0.167 versus 0.875).

Following protection from hunting, the northern elephant seal has recovered to numbers of over 175 000 and it has been removed from the endangered species list (Weber *et al.* [2000](#)). Thus, a population size bottleneck does not necessarily doom a species to immediate extinction. However, the loss of genetic diversity is likely to make it more prone to

extinction from new diseases or other environmental changes. Further, the population will be partially inbred ([Chapter 12](#)), and is likely to have reduced reproductive fitness as a consequence ([Chapter 13](#)). There is a large chance element in the outcome of such bottlenecks. Some situations will be relatively harmless if few deleterious mutations are, by chance, present in the remaining population. In other cases, populations are not so lucky; deleterious mutations are fixed and populations decline to extinction.

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**Table 8.1** *Bottlenecks in endangered species (numbers of founders breeding in captivity)*

Species	Bottleneck size	Reference
Mammals		
Arabian oryx	10	1
Black-footed ferret	7	2
European bison	13	3
Indian rhinoceros	17	3
Père David's deer	~5	4
Przewalski's horse	12 (+1 or more domestic mares)	5

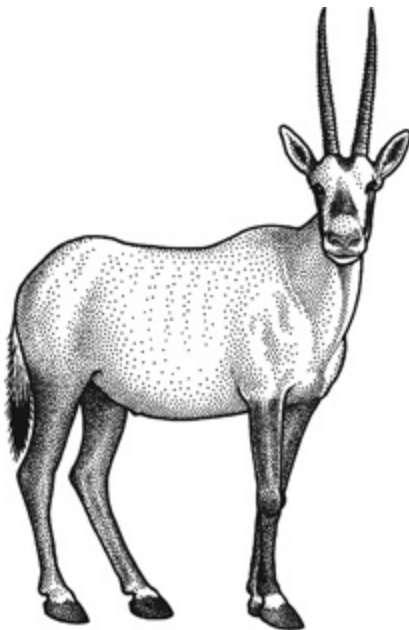
Possible allelic combinations in samples of 2 individuals	Frequency (f)	Heterozygosity ( $H_e$ )	$f \times H_e$
4 $A_1$	$p^4$	0	0
3 $A_1$ :1 $A_2$	$4p^3q$	0.375	$1.5 p^3q$
2 $A_1$ :2 $A_2$	$6p^2q^2$	0.5	$3 p^2q^2$
1 $A_1$ :3 $A_2$	$4pq^3$	0.375	$1.5 pq^3$
4 $A_2$	$q^4$	0	0
Total	1.0		$1.5 pq (p^2 + 2pq + q^2)$ $= 1.5 pq$

Mean heterozygosity in bottlenecked populations  $H_1 = 1.5 pq$

Consequently,  $\frac{H_1}{H_0} = \frac{1.5pq}{2pq} = 0.75 = 1 - \frac{1}{2N}$

Thus, a single-pair bottleneck, on average, reduces heterozygosity by 25% of the initial value.

*References:* 1, Marshall *et al.* (1999); 2, Russell *et al.* (1994); 3, Hedrick (1992); 4, Ballou (1989); 5, Hedrick & Miller (1992); 6, Geyer *et al.* (1993); 7, Ardern & Lambert (1997); 8, Haig *et al.* (1994); 9, Groombridge *et al.* (2000); 10, Wayne *et al.* (1994); 11, Rave *et al.* (1994); 12, Brock & White (1992); 13, Glenn *et al.* (1999).



Arabian oryx



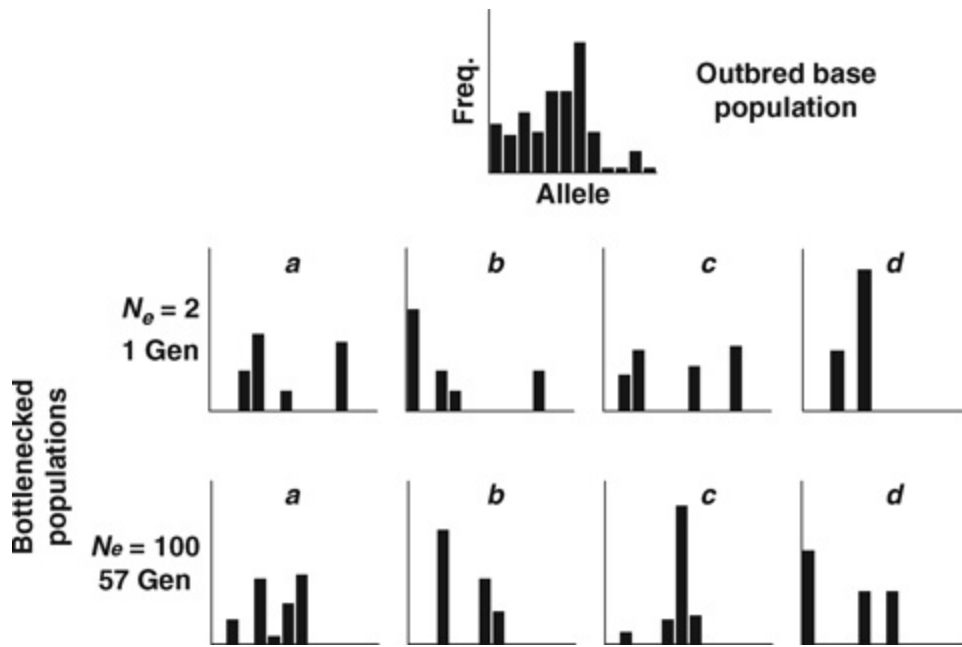
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Population bottlenecks result in loss of alleles (especially rare ones), reduced genetic diversity and random changes in allele frequencies

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Single-pair bottlenecks in experimental populations of fruit flies led to the loss of microsatellite alleles (particularly rare ones), changes in allele frequencies and variation in allele frequencies among replicate bottlenecked populations ([Fig. 8.5](#)). Heterozygosity dropped from 0.61 in the base population to 0.44 in the bottlenecked populations, and the number of alleles declined from 12 to 3.75. Note that the cumulative effects of  $N_e = 100$  over 57 generations has resulted in a similar loss of genetic diversity (see [Chapter 11](#)).



**Fig. 8.5** Effect of single-pair population bottlenecks on experimental populations of fruit flies. The distribution of allele frequencies at a microsatellite locus in the large outbred base population, in four replicate populations subjected to a bottleneck of one pair of flies, and in four populations maintained at a size of 100 for 57 generations (England *et al.* 2003). Alleles are lost, especially rare ones, and allele frequencies distorted in the bottlenecked populations.



Fruit fly

The impact of a single-pair bottleneck on heterozygosity is derived in Table 8.2. Heterozygosity is reduced from  $2pq$  to  $1.5pq$ , a decline of 25%. From that we can generalize to larger-sized bottlenecks, the proportion of initial heterozygosity retained after a single-generation bottleneck being

$$\frac{H_1}{H_0} = 1 - \frac{1}{2N}$$

(8.2)

where  $H_1$  is the heterozygosity immediately after the bottleneck, and  $H_0$  that before. A proportion  $1/(2N)$  of the original heterozygosity is lost. Thus, single-generation bottlenecks have to be severe before they have a substantial impact on heterozygosity. A bottleneck of population size two still retains 75% of initial heterozygosity. The observed decline in heterozygosity from 0.61 to 0.44 following the bottleneck in the experiment described in [Fig. 8.5](#) is in accord with this prediction.

**Table 8.2 Derivation of the effect on heterozygosity of a single-pair, single-generation bottleneck. The heterozygosities are the expected heterozygosities  $H_e$  following the single-pair bottleneck. The base population has two alleles  $A_1$  and  $A_2$  at frequencies  $p$  and  $q$ , respectively (and a heterozygosity of  $2pq$ )**

Possible allelic combinations in samples of 2 individuals	Frequency ( $f$ )	Heterozygosity ( $H_e$ )	$f \times H_e$
4 $A_1$	$p^4$	0	0
3 $A_1$ :1 $A_2$	$4p^3q$	0.375	$1.5 p^3q$
2 $A_1$ :2 $A_2$	$6p^2q^2$	0.5	$3 p^2q^2$
1 $A_1$ :3 $A_2$	$4pq^3$	0.375	$1.5 pq^3$
4 $A_2$	$q^4$	0	0
Total	1.0		$1.5 pq (p^2 + 2pq + q^2)$ $= 1.5 pq$

Mean heterozygosity in bottlenecked populations  $H_1 = 1.5 pq$

Consequently,  $\frac{H_1}{H_0} = \frac{1.5pq}{2pq} = 0.75 = 1 - \frac{1}{2N}$

Thus, a single-pair bottleneck, on average, reduces heterozygosity by 25% of the initial value.

Loss of genetic diversity arises predominantly from sustained reductions in population size, rather than single-generation bottlenecks ([Chapter 11](#)). In the Mauritius kestrel, heterozygosity declined 57% from 0.23 to 0.10 as a result of single-pair bottleneck ([Box 8.1](#)). While this was greater than expected, additional genetic diversity would have been lost during the six generations it

spent at sizes of less than 50.

The impact of a bottleneck on allelic diversity is often proportionately greater, although correlated. Overall, the expected number of alleles ( $A$ ) retained following a single-generation bottleneck is

$$A = n - \sum_{i=1, \text{ alleles}} (1 - p_i)^{2N} \quad (8.3)$$

where  $n$  is the number of alleles before the bottleneck and  $p_i$  is the frequency of the  $i$ th allele. The  $\Sigma$  term is the number of alleles lost. Example 8.2 uses this equation to predict the loss of alleles due to a bottleneck of two individuals for the fruit fly microsatellite locus illustrated in [Fig. 8.5](#).

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### **Example 8.2 Predicted and observed effects of a single-pair bottleneck on heterozygosity and allelic diversity**

For the fruit fly bottleneck experiment in [Fig. 8.5](#), the base population had an initial heterozygosity of 0.61. The predicted effects of a single-pair bottleneck ( $N = 2$ ) is

$$H_1 = H_0 \left(1 - \frac{1}{2N}\right) = 0.61 \left(1 - \frac{1}{2 \times 2}\right) = 0.46$$

The observed heterozygosity in the bottlenecked fly populations was 0.44, close to this expectation.

The base population had 11 alleles at the DMU1951 microsatellite locus with alleles at frequencies of 0.067, 0.067, 0.090, 0.157, 0.157, 0.149, 0.097, 0.142, 0.007, 0.060 and 0.007 (data slightly different from that in [Fig. 8.5](#)). Using [Equation 8.3](#), we predict the number following a single-pair bottleneck as:

$$A = n - \sum_{i=1, \text{ # alleles}} (1 - p_i)^{2N} = 11 - [0.933^4 + 0.933^4 + 0.910^4 + 0.843^4 + 0.843^4 + 0.851^4 + 0.903^4 + 0.858^4 + 0.993^4 + 0.940^4 + 0.993^4] = 11 - 7.67 = 3.33$$

The observed number of alleles in the bottlenecked populations was 3.57, in good agreement with the theoretical prediction.

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## Effect of population bottlenecks on quantitative genetic diversity

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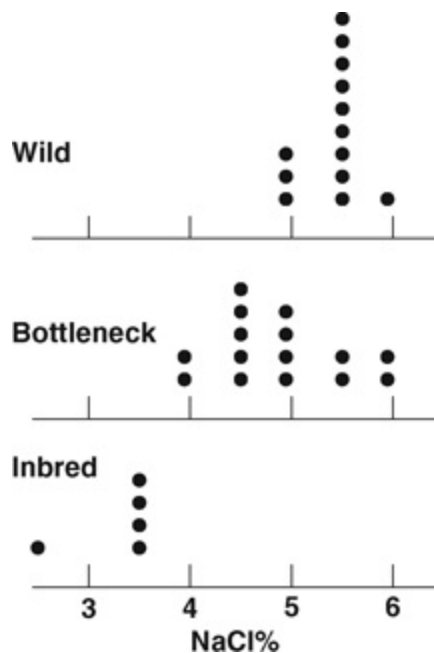
Population bottlenecks reduce evolutionary potential

For quantitative characters showing only additive genetic variation, the expected loss of quantitative genetic variation due to a bottleneck is also a  $1/(2N)$  proportional reduction in variation. This expectation has been verified in several selection experiments in fruit flies (Frankham [1980](#)).

The situation is more complex for characters exhibiting non-additive genetic variation, as bottlenecks can actually increase additive genetic variation due to increased homozygosity for rare recessive alleles (Robertson [1952](#)). Such increases have been observed in bottlenecked populations for characters exhibiting non-additive variation (Briggs & Goldman [2006](#); van Buskirk & Willi [2006](#); Willi *et al.* [2006](#)). However, their relevance to evolutionary potential is questionable as the mean values for the characters drop due to the inbreeding involved. Direct tests of the impact of population bottlenecks on evolutionary potential in fruit flies and canola plants both



found clear reductions due to the bottleneck (Briggs & Goldman 2006) (Fig. 8.6).

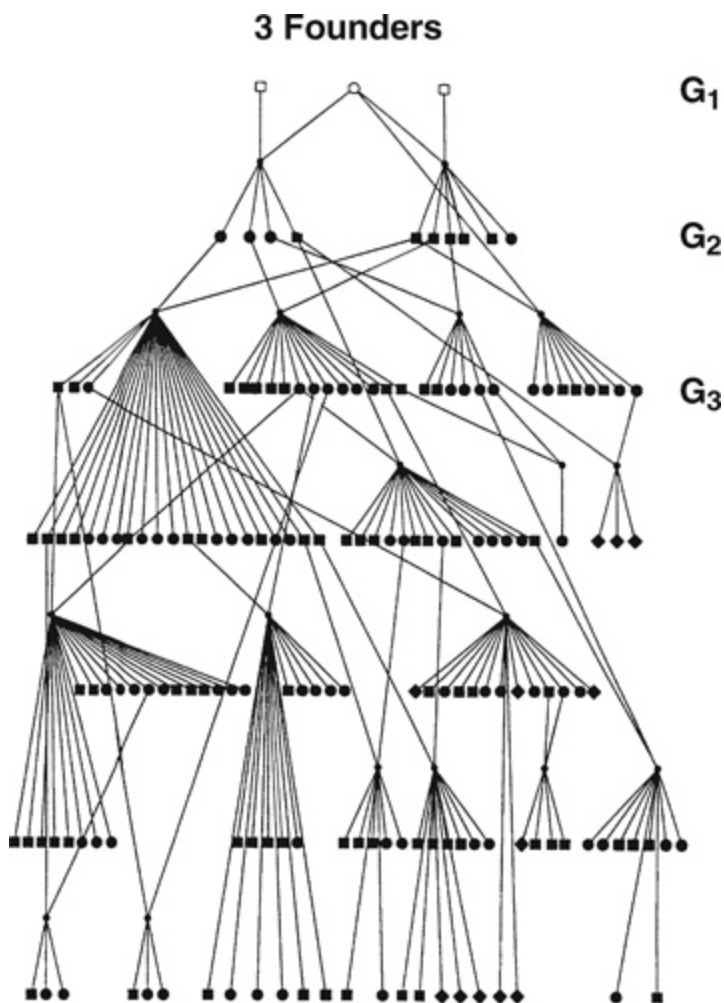


**Fig. 8.6** Effects of population bottlenecks on evolutionary potential in fruit flies (Frankham *et al.* 1999). Populations were subjected to a single-pair bottleneck for one generation. These populations, their base population, and highly inbred (homozygous) populations from the same stock were all increased to the same population size, placed in cages and subjected to increasing concentrations of NaCl until extinction. *Extinctions occurred earlier in the bottlenecked populations and their timing was more variable than in the wild base population.*

## Inbreeding

Inbreeding is unavoidable in small closed random mating populations and leads to reductions in reproduction and survival

Every individual beyond the second generation in the Mexican wolf pedigree in Fig. 8.7 has parents that are related. Inbreeding also becomes inevitable in larger populations, but it takes longer. For example, a population of size 100 over 57 generations becomes, on average, as inbred as the progeny of a brother–sister mating (Chapter 11).



**Fig. 8.7** Inbreeding is unavoidable in small populations. Pedigree for the Certified population of Mexican wolves (from Hedrick, Miller, Geffen & Wayne, © 1997 *Zoo Biology*, **16**: 47–69, reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley and Sons, Inc.). Square, males; circles, females; diamonds, unknown sex. *Within a few generations parents of all individuals share common ancestors, i.e. progeny are inbred.*

Inbreeding is of profound importance in conservation biology as it leads to

reductions in heterozygosity, to reduced reproduction and survival (inbreeding depression) and to increased risk of extinction ([Chapters 2 and 13](#)).



Mexican wolf

## Measuring population size

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The population size relevant to genetic impacts is usually smaller than the census population size

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So far, we have discussed the impacts of size on genetic processes within populations as though these are related to the absolute or census size. This is rarely the case. There are many situations where apparently large ‘safe’ populations may be reduced to levels where they are genetically endangered. For example, many populations of small mammals, insects and coral reef organisms fluctuate wildly in size, and it is the minimum size that most profoundly affects genetic processes (see [Chapter 11](#)). Most populations contain juveniles, steriles and post-reproductives that should not be counted in the reproductive population size. Amongst reproductives, there may be considerable variation in their contributions to subsequent generations. Further, the genetic consequences of monogamy versus harems differ, as does random mating versus selfing versus asexual reproduction.

The same number of individuals may result in very different genetic impacts, with genetically effective population sizes in different species depending on population structure and breeding system. Consequently, we must define precisely what we mean by population size in conservation genetics. We do this by comparing real populations to a hypothetical **idealized population**. Conversion of census population size to an effective population size provides a common yardstick that allows comparisons and predictions to be extended over species with a diversity of structures and breeding systems.

## The idealized population

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The idealized population, to which all other populations are compared, is a closed random mating population of hermaphrodites with discrete generations, constant population size, and random (Poisson) variation in family sizes

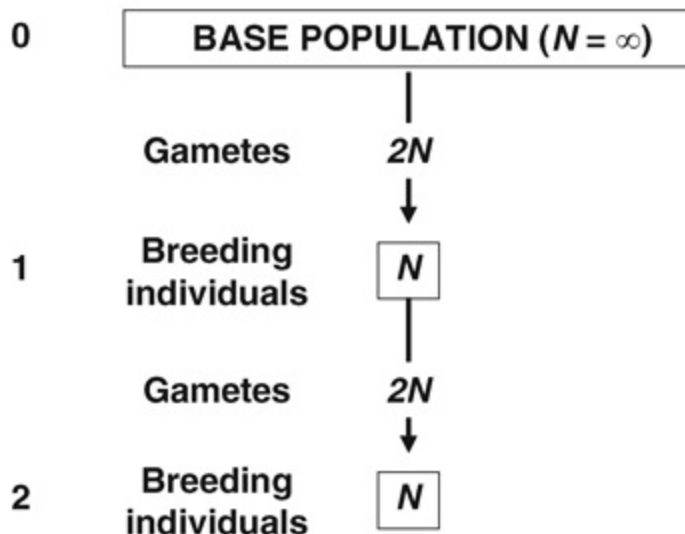
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We begin by assuming a large (essentially infinite) random mating base population, from which we take a sample of size  $N$  adults to form the ideal population ([Fig. 8.8](#)). This population is maintained as a random mating, closed population in succeeding generations. Alleles may be lost by chance, and allele frequencies may fluctuate due to genetic drift. The simplifying conditions applying to the idealized population are:

- the number of breeding individuals is constant in all generations
- generations are distinct and do not overlap
- there is no migration or gene flow
- all adult individuals are potential breeders
- all individuals are hermaphrodites (possess both female and male sex organs)

- union of gametes is random, including the possibility of selfing
- there is no selection at any stage of the life cycle
- mutation is ignored
- the average number of gametes contributed per potentially breeding individual to potential breeders of the next generation is 2, and this has a variance of 2.

#### Generation



**Fig. 8.8** Idealized population. From the very large base population a sample of  $N$  adults is taken and this population is maintained as a random mating, closed population with constant number of parents in each generation.

Within the population, breeding individuals contribute gametes equally to a pool from which zygotes are formed. Survival of zygotes is random, so that the contributions of adults to the next generation are unequal. The mean number of offspring per adult is 1, but varies randomly from 0, 1, 2, 3, 4, etc. for different individuals, according to the terms of the **Poisson distribution**.

Because of these assumptions, the characteristics of the idealized population are well defined and a large body of theory has been derived for them. Consequently, by equating real populations to the idealized population, theory can be utilized to make practical predictions.

### Effective population size ( $N_e$ )

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Genetic processes in small populations depend on the effective population size rather than on the number of individuals

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We can standardize the definition of population size by describing a population in terms of its **effective population size** ( $N_e$ ). The effective size of a population is the size of an idealized population that would lose genetic diversity (or become inbred, or drift) at the same rate as the actual population. For example, if a real population loses genetic diversity at the same rate as an ideal population of 100, then we say the real population has an effective size of 100, even if it contains 1000 individuals. Thus, the  $N_e$  of a population is a measure of its genetic behaviour, relative to that of an ideal population.

All of the adverse genetic outcomes of small population size depend on the effective population size, rather than on the absolute number of individuals. In practice, the effective size of a population is usually much less than the number of breeding adults. Real populations deviate in structure from the assumptions of the idealized population by having females and males, unequal sex ratios, non-random mating, high variation in family sizes, variable numbers in successive generations, and overlapping generations. Details of how to calculate  $N_e$  are given in [Chapter 11](#). For the time being, we shall simply recognize that it is the effective size  $N_e$ , and not the actual number of individuals ( $N$ ), that should be used in most equations (e.g. 8.1–8.3).

## Selection in small populations

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Selection is less effective in small than large populations

Large populations show greater adaptive evolutionary capabilities than small, endangered populations. Selection operating on body colour in the red flour beetle was more consistent in its effect in large than in small populations ([Fig. 8.2](#)). Despite selection against the black allele, one small population became homozygous for this deleterious allele. This provides a critical insight for conservation genetics: selection is less effective in small than in large populations.

Small populations show less response to directional selection for quantitative characters than larger populations

Small populations lose genetic diversity each generation; therefore selection response should be reduced compared to large populations. From a model of this process, Robertson ([1960](#)) predicted that the total amount of selection response (the limit) to directional selection would depend on the product of effective population size and the selection differential. These predictions have been verified for several different quantitative characters in a range of species including fruit flies, mice, chickens and maize ([Weber 2004](#)).

The evolutionary potential of endangered species is seriously compromised, compared to non-endangered species, as they have less initial genetic diversity ([Chapter 3](#)) and they lose genetic diversity at a greater rate

in each generation ([Chapter 15](#)).

---

Deleterious alleles are more likely to be fixed in small than in large populations

---

An important implication of the lower efficiency of selection in small than in large populations is that deleterious alleles are less likely to be removed by natural selection and may even become fixed ([Fig. 8.2](#)). This can lead directly to reduction in reproductive fitness and increased extinction risk ([Chapters 13 and 15](#)).

## **Mutation–selection equilibrium in small populations**

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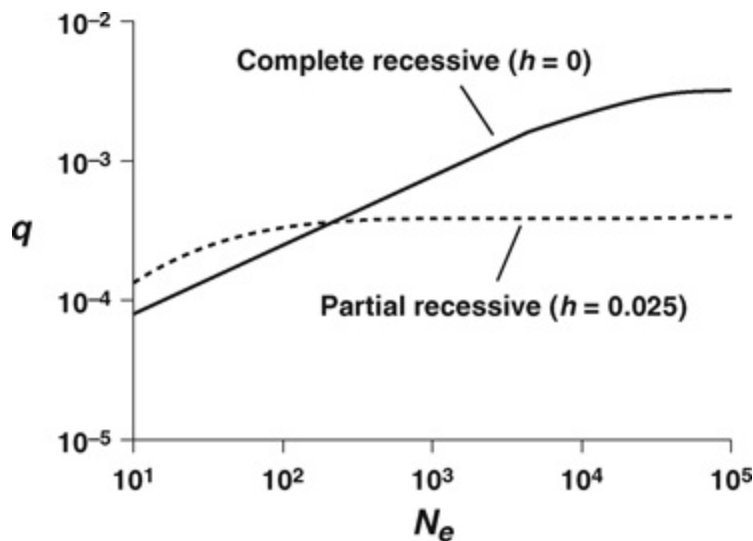
Equilibrium frequencies for deleterious alleles are, on average, lower in small populations than in large populations

---

Mutation–selection balance maintains deleterious mutations in populations at low frequencies at many loci ([Chapter 7](#)). However, alleles are lost by drift in finite (small) populations, especially rare mutations. Further, recessive alleles, whose frequencies increase by drift, are more effectively reduced by selection. Consequently, the equilibrium frequencies for deleterious alleles



are, on average, lower in small populations than in large populations (Fig. 8.9). For example, the expected equilibrium frequencies for a recessive lethal allele with a mutation rate of  $10^{-5}$  is 30-fold lower in populations of size 10 as opposed to very large populations. The equilibrium frequency for a recessive lethal of  $\sqrt{u}$  (Equation 7.4) only applies when  $N_e$  is at least 1 million (Wright 1969). The relationship between equilibrium frequency and population size is weaker for partially recessive lethals. For example, a partially recessive lethal, with a 2.5% decrement in heterozygote fitness, has an equilibrium frequency of  $4 \times 10^{-4}$  in a very large population, and about  $10^{-4}$  in a population with an effective size of 10.



**Fig. 8.9** Equilibrium frequencies for recessive lethal alleles in populations of different sizes. A mutation rate of  $10^{-5}$  is assumed. The solid line is for a completely recessive lethal, and the dashed line for a partially recessive lethal allele that reduces reproductive fitness by 2.5% in heterozygotes (after Crow & Kimura 1970).

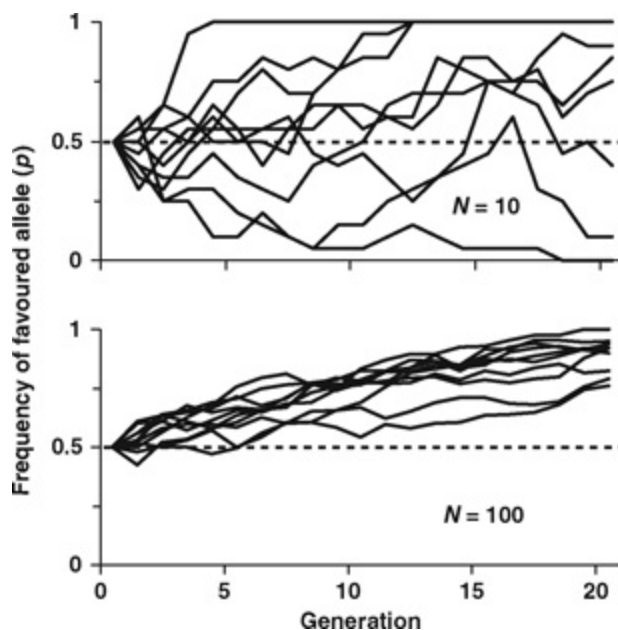
The variance in deleterious allele frequencies will be high in small populations. Many loci will have no deleterious alleles, but some will have relatively high frequencies by chance, as we saw for chondrodystrophy in California condors (lethal allele frequency of ~17%; Chapter 4). This allele is expected to have a mutation–selection equilibrium frequency of about 0.3% in a large population (Chapter 7). Other endangered species have been found to have elevated frequencies of alleles causing genetic diseases (Table 7.3),

as do bottlenecked human populations (Diamond & Rotter 1987).

## Computer simulation

Computer simulation is used to investigate problems that are difficult to solve mathematically, such as the impact of selection or mutation in small populations

Due to the stochastic nature of genetic drift, the impacts of selection on allele frequencies in small populations are difficult to model algebraically. Consequently, computer simulations are often used to study the impacts of chance and selection (Box 8.3). For example, the flour beetle experiment described in Fig. 8.2 has been simulated in Fig. 8.10. Note the similarity of the results with the experimental data.



**Fig. 8.10** Computer simulation illustrating the operation of selection in

replicate populations with sizes of  $N = 10$  and  $N = 100$ . Selection is for an additive model with  $s = 0.1$ . These simulations were conducted using a random number generator in spreadsheet software.

---

### **Box 8.3 Complex models and computer simulation**

The joint impacts of chance with selection, migration and mutation rapidly becomes too complicated to investigate using algebraic models. These are investigated either by using relatively complex mathematics, such as diffusion equations (Crow & Kimura 1970), or by building stochastic computer models. Computer models that include chance are called Monte Carlo simulations, after the famous casinos in that principality. Monte Carlo simulations yield a distribution of outcomes, rather than a single outcome, as shown in [Fig. 8.10](#).



Computer simulations are used in several different ways in conservation genetics. They may be used to:

- verify the results of mathematical models
- provide numerical solutions for expressions produced by stochastic mathematical models
- check the validity of approximate mathematical solutions to problems
- suggest a solution to a problem that may subsequently be solved mathematically
- provide a 'null' hypothesis against which empirical results may be compared

- investigate problems that are too complex to solve with mathematical models.

In the latter context, computer simulation provides links between simple tractable mathematical models (with many simplifying assumptions) and experiments with real living organisms in all their complexity. Computer simulations can incorporate sufficient complexity to realistically address most problems. For example Lacy (1987) used computer simulations to evaluate the likely effects of drift, selection, migration and population subdivision on small populations of endangered species. For complex pedigrees, the computation of probabilities that alleles are lost, or retained, over time are typically determined using ‘gene drop’ computer simulations (MacCluer *et al.* 1986). Further, Ballou & Lacy (1995) used computer simulation to evaluate the effects on retention of genetic diversity and inbreeding of alternative genetic management schemes proposed for endangered species. Their simulations followed many replicates over several generations for a single locus, based on starting populations with different pedigrees. Their work led to a new procedure (minimizing mean kinship) being instituted for genetic management of captive populations (Chapter 19). Despite a number of simplifying assumptions, their computer predictions were subsequently verified in experiments with fruit flies.

More complex computer models are used to assess extinction risk due to all important threatening processes – a procedure called population viability analysis (Fig. 2.4; Chapter 22).

---

## Summary

1. Populations of conservation concern are small or declining.
2. Evolution in small populations involves a greater impact of chance, and more inbreeding, than in large populations.
3. Chance effects (genetic drift) arise from random sampling of gametes.
4. Genetic drift results in random fluctuations in allele frequencies,

diversification among replicate populations, fixation and loss of genetic diversity.

5. The genetic consequences of small populations depend upon the effective population size, rather than on the actual number of individuals.
6. Selection is less effective in small than in large populations.
7. The equilibrium frequencies for deleterious alleles due to mutation–selection balance are generally lower in small than in large populations.

### Further reading

Crow & Kimura (1970) *Introduction to Population Genetics Theory*. Relatively advanced treatment of evolutionary theory in small populations.

Falconer & Mackay (1996) *Introduction to Quantitative Genetics*. Chapter 3 provides a very clear introduction to the topics in this chapter.

Hartl & Clark (2007) *Principles of Population Genetics*. Covers topics of this chapter.

Hedrick (2005a) *Genetics of Populations*. Has a more extensive treatment of many of the topics in this chapter.

Willi *et al.* (2006) Excellent review on the impacts of small population size in reducing ability to adapt.

### Software

BOTTLENECK: Software to test for a signal of a population bottleneck from allele frequency data (Piry *et al.* 1999).  
[www.montpellier.inra.fr/URLB/bottleneck/bottleneck.html](http://www.montpellier.inra.fr/URLB/bottleneck/bottleneck.html)

DRIFT: A free animation of how alleles are passed from one generation to the next. <http://nationalzoo.si.edu/goto/Ballou/>

EASYPOP: Software to simulate the effects of mutation, population size,

drift, migration and population fragmentation (Balloux 2001).  
[www.unil.ch/dee/page36926\\_fr.html](http://www.unil.ch/dee/page36926_fr.html)

SELECTION: Software to simulate the effects of selection, drift, mutation and migration on a single locus. [www.gsoftnet.us/GSoft.html](http://www.gsoftnet.us/GSoft.html)

## Problems

- 8.1 Probability of chance loss of an allele. If a heterozygous brain coral with genotype  $A_1A_2$  has three offspring by selfing, what is the probability that allele  $A_1$  is absent in the offspring?
- 8.2 Probability of chance loss of an allele. If two Siberian tiger parents with genotypes  $A_1A_2$  and  $A_1A_3$  have four offspring, what is the probability that allele  $A_1$  is absent in the offspring? That  $A_2$  is absent in the offspring? That  $A_3$  is absent in the offspring?
- 8.3 Probability of chance loss of an allele. If a Guam rail population has two alleles  $A_1$  and  $A_2$  at frequencies of 0.9 : 0.1, respectively, (a) what is the probability that  $A_2$  is lost in the subsequent generation if 12 offspring are produced? (b) What is the probability that  $A_2$  is lost in the subsequent generation if 100 offspring are produced? Compare (a) and (b).
- 8.4 Probability of retaining alleles under random sampling. How many offspring would be needed to be 95% certain that the  $A_1$  allele was sampled from the first individual in the top line of the cheetah pedigree in [Fig. 8.1](#)?
- 8.5 Loss of genetic diversity. What proportion of the initial heterozygosity is retained following a single-generation bottleneck in (a) a single plant of *Pritchardia munroi*, an endemic Hawaiian palm? (b) Chatham Island black robins reduced to five individuals? (c) whooping cranes reduced to 14 individuals? (d) Indian rhinoceros population in Chitwan, Nepal reduced to about 70 individuals? (e) southern bluefin tuna reduced to 300 000 individuals?
- 8.6 Loss of allelic diversity. What is the probability that an allele with

an initial frequency of  $q$  is lost following a single-generation bottleneck when  $q = 0.1$  in (a) one plant of giant red Indian paintbrush? (b) in two Mauritius kestrels? (c) in 50 northern hairy-nosed wombats? (d) when  $q = 0.05$  in 25 Siberian tigers?

## Practical exercises: Computer simulations

The following computer simulation exercises are designed to assist readers to understand aspects of the evolution in small populations. Those involving drift alone can be completed using a package such as EASYPOP, while SELECTION can be used for cases involving drift and selection.

1. Genetic drift and diversification. Compare the proportion of populations (i) still polymorphic, (ii) going to fixation ( $q = 1$ ) and (iii) losing the  $A_2$  allele ( $q = 0$ ) in populations of different sizes (10 vs. 50) over 100 generations, beginning at frequencies of  $q = 0.5$  and  $q = 0.1$ .

These require 50–100 replicates and can be done individually, or compiled as the sum of replicates from all the students in a class.

Simulation	Fixed $q = 1$	Polymorphic	Lost $q = 0$
$q_0 = 0.5$ $N = 50$			
$q_0 = 0.5$ $N = 10$			
$q_0 = 0.1$ $N = 50$			
$q_0 = 0.1$ $N = 10$			

How do the results differ with (a) population size, and (b) with different starting frequencies?

2. Adaptive evolution with strong selection. Industrial melanism in peppered moths (favoured dominant allele). Industrial melanism increased in frequency from an allele frequency  $q = 0.005$  in 1848 to about 0.90 in 1900 (52 generations). The relative fitnesses are approximately as follows:

Typical $tt$	Melanic $Mt$	Melanic $MM$
0.7	1	1

Simulate this case for 100 generations with  $N = 50$  and record the results in the table below.

Simulation	Fixed $q = 1$	Polymorphic	Lost $q = 0$
$q_0 = 0.005$ $N = 50$			

In what proportion of cases was the deleterious allele fixed?

Compare these results with the deterministic case from [Chapter 6](#).

- Adaptive evolution with weak selection. For a partially dominant locus with following fitnesses (akin to an allozyme locus or a MHC allele), simulate changes in allele frequencies over 2000 generations with  $N = 50$  and  $N = 10$ , beginning with  $q = 0.1$ .

$A_1A_1$	$A_1A_2$	$A_2A_2$
0.99	0.992	1.0

Record your results in the table below:

Simulation	Fixed $q = 1$	Polymorphic	Lost $q = 0$
$q_0 = 0.1$ $N = 50$			
$q_0 = 0.1$ $N = 10$			

In what proportion of cases was the deleterious allele fixed?

Compare these results with the deterministic case by running the same fitnesses and starting frequencies.



## Chapter 9 Maintenance of genetic diversity

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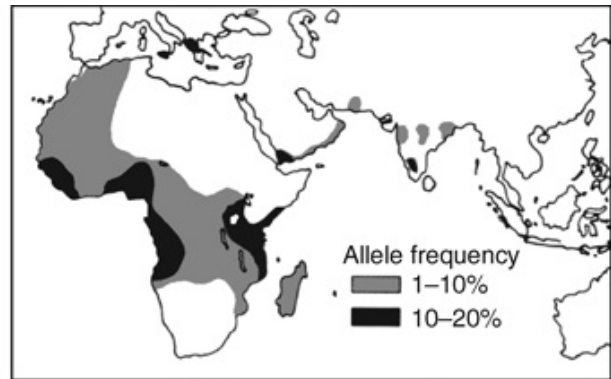
Genetic diversity in populations is maintained through natural processes including equilibria between neutral mutation and random genetic drift, deleterious mutation and selection, and balancing selection. Balancing selection generally impedes the loss of genetic diversity. The principal mechanisms maintaining genetic diversity differ among characters and between small versus large populations

### Terms

Associative overdominance, balancing selection, effectively neutral, frequency-dependent selection, heterozygote advantage, intron, inversions, neutral mutation, non-synonymous substitutions, overdominance, pseudogene, rare advantage selection, selectively neutral, synonymous substitutions, transient polymorphisms, trans-species polymorphism



(a) *Falciparum* malaria



(b) Sickle-cell anaemia



(c) Thalassaemia



(d) G6pd deficiency

Malaria and genetic diversity in humans. Distribution of *falciparum* malaria (a) and the frequencies of alleles that confer resistance to the disease, (b) sickle-cell anaemia, (c) thalassaemia and (d) G6pd deficiency (after Strickberger 1985, based on Allison 1961)

## Conservation of genetic diversity

Maintenance of genetic diversity is a major objective in conservation

programs, as genetic diversity is required for populations to evolve

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Conservation biologists need to understand how genetic diversity is maintained through natural processes, if conservation programs are to be designed for its maintenance in managed populations of endangered species.

Populations vary in their levels of genetic diversity ([Chapter 3](#)). Most large, widespread species have high levels of genetic diversity. Conversely, smaller populations, island populations and endangered species often display much lower levels. These differences are a direct result of the interacting processes of selection, genetic drift, mutation and migration operating within particular breeding systems. The level of diversity therefore depends on which process predominates. This can vary for different characters and is modified in small versus large populations. In general, genetic drift predominates over deterministic mechanisms (such as selection) in smaller populations.

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There are two main hypotheses for maintenance of genetic diversity for alleles at intermediate frequencies: neutral mutations undergoing random genetic drift, and balancing selection

---

Before considering the contributions of balancing selection and neutral mutation–random genetic drift to maintenance of genetic diversity, we need to consider the selective values associated with different classes of mutations.

## Fate of different classes of mutations

The major classes of mutations are:

- deleterious mutations
- beneficial mutations
- neutral mutations
- mutations whose effects are favoured in some circumstances, but not in others ([Chapter 7](#)).

The fate of each of these is considered below.

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The majority of newly arisen mutations are deleterious. Deleterious mutations are removed by selection but continue to be added by mutation

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While we do not know the precise proportions of the four types of mutations, there is no doubt that those with effects on the phenotype are overwhelmingly deleterious. Deleterious mutations are continually removed by selection. The balance between mutation and selection generally keeps deleterious alleles at very low frequencies ([Chapter 7](#)).

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Beneficial mutations are fixed by natural selection

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A (very) small proportion of mutations is beneficial. These mutations increase in frequency until they reach fixation, provided they are not lost by chance when rare in early generations. Loci with such alleles will be observed as polymorphic during the phase when the alleles are rising in frequency prior to reaching fixation (**transient polymorphisms**). Loci with such mutations will only rarely be observed as polymorphic as they represent a minority class of mutations and the beneficial alleles are fixed relatively rapidly, at a rate dependent upon their selective advantage.

---

Many mutations outside functional regions and some within them are neutral

---

A proportion of mutations is **neutral** (Fig. 7.1), i.e. they have the same impact on reproductive fitness as pre-existing alleles. Many mutations in untranslated DNA (regions between loci, and introns) and DNA base substitutions that do not result in amino acid changes (synonymous substitutions) are expected to fall into this category. The fate of neutral mutations is determined by genetic drift alone and they may also create transient polymorphisms. They do not, therefore, enter into our discussion of the maintenance of genetic diversity for reproductive fitness. Their fate depends entirely on population size and the rate of neutral mutations.

---

Mutations subject to any form of balancing selection are actively retained in large populations

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The fourth class of mutations is favoured by selection in some circumstances, and selected against in others. This is termed **balancing selection**. These alleles are maintained in the population at relatively intermediate frequencies, resulting in polymorphisms.

There are three primary forms of balancing selection. Some alleles are advantageous in heterozygotes and disadvantageous in homozygotes (**heterozygote advantage** or **overdominance**), while others are advantageous when rare, and disadvantageous when common (**rare allele advantage** or **frequency-dependent selection**). Finally, some of these mutations have selective values that are advantageous in some environments and disadvantageous in others conditions, e.g. one season versus another, or one environmental niche versus another. Such alleles display genotype  $\times$  environment interactions ([Chapter 5](#)). (Mutation–selection balance is not described as balancing selection.)

---

Most of the genetic diversity observed in populations is likely to represent neutral alleles and alleles subject to balancing selection

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Since both deleterious and favourable mutations are lost or rapidly go to fixation, the polymorphisms with intermediate allele frequencies observed in natural populations primarily represent alleles that are neutral together with those maintained by balancing selection.

## Maintenance of genetic diversity in large populations

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Selection has a relatively greater role in maintenance of genetic diversity in larger populations, while drift is more important in small populations

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Our discussion of the maintenance of genetic diversity in large populations begins by considering the fate of neutral mutations under genetic drift, considers the extent of selection on different loci and characters, discusses balancing selection in large populations and concludes with maintenance of genetic diversity for fitness characters. Maintenance of genetic diversity in small populations (i.e. those of most conservation concern) is treated primarily in the latter part of the chapter so that we can compare and contrast it with the situation for large populations.

## Neutral mutations under random genetic drift

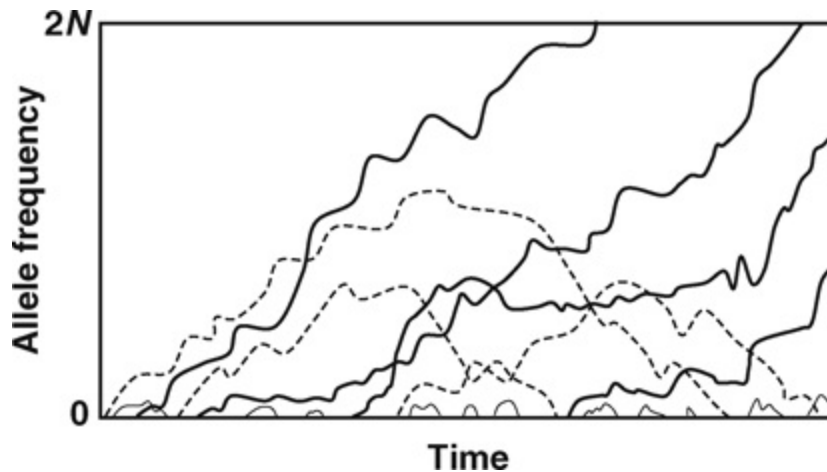
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A proportion of the genetic diversity in natural populations is due to neutral mutations whose fate is determined by random genetic drift

---

Most neutral mutations are lost within a few generations of origin (because they start at very low frequencies). However, new mutations continue to be produced. A small proportion rise in frequency just by chance, and some go

to fixation (Fig. 9.1). As a *de novo* mutation has an initial frequency of  $1/2N_e$ , this proportion is  $1/2N_e$ . The flux of these alleles is such that, at any one time, some loci are likely to be polymorphic. This is referred to as the **neutral theory** of molecular evolution.



**Fig. 9.1** Neutral mutation – random genetic drift (after Crow & Kimura 1970). The figure illustrates the flux of neutral mutations over a very large number of generations in a very large population. Most mutants are lost within a few generations (thin lines). Occasional mutants increase in frequency. Some of these increase to eventual fixation (heavy lines), while others are lost (dotted lines). *At any point in time there are polymorphic loci (transient polymorphisms).*

Evolution in non-coding DNA is primarily due to neutral mutation – random genetic drift (especially in threatened species), while its role in protein evolution is more controversial (Kimura 1983; Chapter 10).

Several lines of evidence supporting the neutral theory are given below and most of these are not predicted by alternative hypotheses (Kimura 1983).

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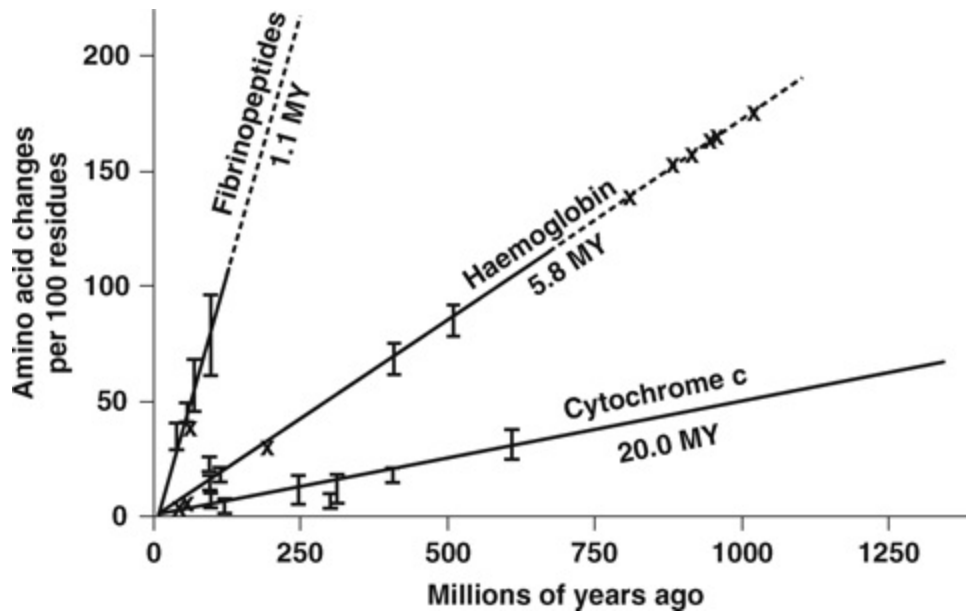


The neutral theory predicts a constant rate of molecular evolution, regardless of population size

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The derivation of the relationship between rate of molecular evolution and neutral mutation rate is as follows. The number of neutral mutations per generation in a population of size  $N_e$  is  $2N_e u$ , where  $u$  is the neutral mutation rate. However, the probability of fixing a neutral mutation is its initial frequency,  $1/2N_e$ . Consequently, the rate of substitution (i.e. one allele replacing another) at equilibrium is the product of these two values  $2N_e u \times 1/2N_e = u$ . Thus, the rate of molecular evolution is expected to be constant in different-sized populations and to equal the neutral mutation rate. This derivation leads to constancy per generation. However, Kimura has argued that the rate should be constant per year, as the number of germ line generations is approximately constant per year, regardless of generation length. Whether the rate of molecular evolution should be constant per year or per generation remains a matter of controversy, but it is closer to constant over generations than years in mammals (Nikolaev *et al.* 2007).

The evidence favours an approximate constancy in rates of amino acid substitution in proteins (Fig. 9.2) (Kimura 1983, but see Gillespie 1991). Different rates are expected for proteins with different degrees of functional constraint, as they have different neutral mutation rates.



**Fig. 9.2** Rate of amino acid substitutions is approximately constant as predicted by the neutral theory, but differs among proteins (after Hartl & Clark 2007).

The neutral theory predicts higher levels of polymorphism for regions of the genome subject to less functional constraints

As predicted, polymorphism in non-translated DNA (introns and pseudogenes – non-functional loci) and for **synonymous mutations** (ones that do not alter the amino acid sequence of proteins) is higher than in regions of DNA with obvious function (Hartl & Clark 2007). Further, levels of protein polymorphism are related to the size of proteins, as predicted by the neutral theory (Ward *et al.* 1992). Larger proteins are expected to generally have higher neutral mutation rates than smaller ones.

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The neutral theory predicts that there will be a positive relationship between genetic diversity and population size

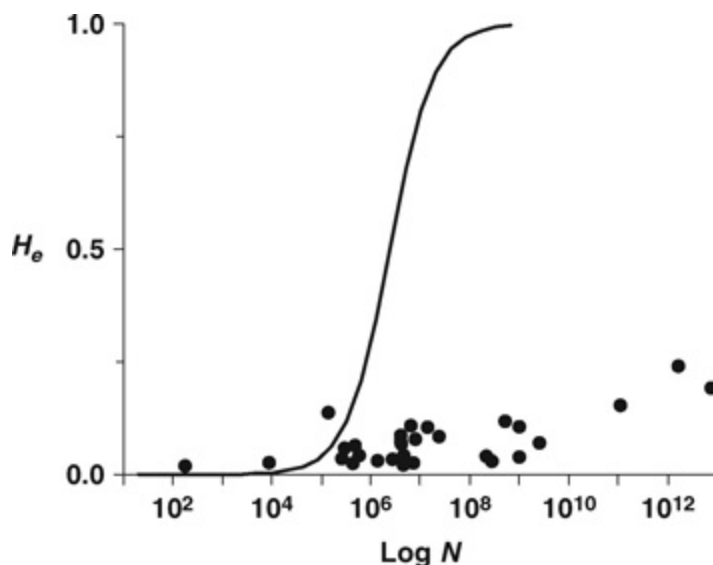
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Under the neutral theory, the balance between mutation adding alleles and drift removing them determines levels of genetic diversity. Since alleles drift to fixation more rapidly in small than in large populations, neutral theory predicts that the expected heterozygosity ( $H_e$ ), and effective number of alleles ( $n_e$ ) will be higher in larger than in smaller populations:

$$H_e = \frac{4N_e u}{(4N_e u + 1)} \quad (9.1)$$

$$n_e = 4N_e u + 1 \quad (9.2)$$

The expected relationship between genetic diversity and population size is shown in [Fig. 9.3](#), and illustrated with numerical cases in Example 9.1.



**Fig. 9.3** Predicted relationship between heterozygosity and population size according to the neutral theory, and the experimentally determined relationship (after Nei 1987). The curve is the predicted relationship with a neutral mutation rate of  $10^{-7}$ , and dots are observed data points. *Observed heterozygosities increase with population size, but at a much slower rate than predicted by the neutral theory.*

The neutral mutation rate is lower than the overall mutation rate as it excludes deleterious and favourable mutations. A typical rate is  $10^{-9}$  per nucleotide site per year, but it varies widely for different regions according to the functional constraints on the molecule (Kimura 1983).

While there is strong evidence for a relationship between genetic diversity and population size both among and within species (Frankham 1996; Leimu *et al.* 2006; Chapter 11), the shape of the relationship is not of the form predicted by the neutral theory (Fig. 9.3). The observed relationship also does not conform to that predicted by balancing selection, but is compatible with a model of near neutral mutations, as described below.

---

### **Example 9.1 Predicted heterozygosities and effective number of alleles in different sized populations according to the neutral theory**

Let us consider two populations both with neutral mutation rates of  $3 \times 10^{-6}$  for a locus, one with an effective size of 100 and the other of 10 million. Heterozygosity for the smaller population is predicted to be

$$H_{\text{small}} = \frac{4N_e u}{(4N_e u + 1)}$$

$$= \frac{(4 \times 100 \times 3 \times 10^{-6})}{[(4 \times 100 \times 3 \times 10^{-6}) + 1]} = 1.2 \times 10^{-3}$$

while that for the larger populations is

$$H_{\text{large}} = \frac{(4 \times 10^7 \times 3 \times 10^{-6})}{[(4 \times 10^7 \times 3 \times 10^{-6}) + 1]} = 0.99$$

The effective number of alleles in the smaller population will be

$$n_{\text{e-small}} = 4N_e u + 1 = (4 \times 100 \times 3 \times 10^{-6}) + 1 = 1.0012$$

while that in the larger population is expected to be

$$n_{\text{e-large}} = (4 \times 10^7 \times 3 \times 10^{-6}) + 1 = 121$$

Consequently, a stable population of 10 million is expected to have much greater heterozygosity and allelic diversity than a population of 100.

A purely neutral theory does not adequately explain molecular evolution, or genetic diversity, and it does not apply to fitness characters

There are good reasons to reject a purely neutral theory of molecular evolution (see Kreitman & Asahi 1995; Hey 1999; Nei 2005). The near neutral theory, that encompasses both strictly neutral and mildly deleterious alleles, is considered more plausible (Ohta 1996; Hartl & Clark 2007). With this theory there is a flux of mutations entering populations and being lost, or fixed, as for the neutral theory. However, only a small proportion is neutral and many more are very mildly deleterious mutants (Fig. 7.1). Most mildly deleterious alleles are removed from large populations by selection, but they are effectively neutral in small populations. The near neutral theory probably

explains a substantial proportion of genetic variation for characters and DNA sequences subject to little selection (see below). For example, evolutionary patterns for non-synonymous sites in humans are in agreement with the expectations of the near neutral theory (Nikolaev *et al.* 2007).

However, neither neutral or near neutral theories apply to all loci. There is clear evidence for balancing selection (see below) on some protein polymorphisms, on the MHC and self-incompatibility loci, on the sex locus in haplo-diploids, on many inversion polymorphisms and on most visual polymorphisms (Hoffmann *et al.* 2004; Charlesworth 2006; Hoekstra 2006). The neutral theory clearly does not apply to genetic diversity for reproductive fitness, a major conservation focus.

## **Selection intensities vary among characters**

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The selective forces on different characters vary, being very weak or absent for untranslated DNA, weak for most translated DNA and protein polymorphisms, stronger for inversions, MHC and self-incompatibility loci clusters, and strongest for visual polymorphisms and reproductive fitness

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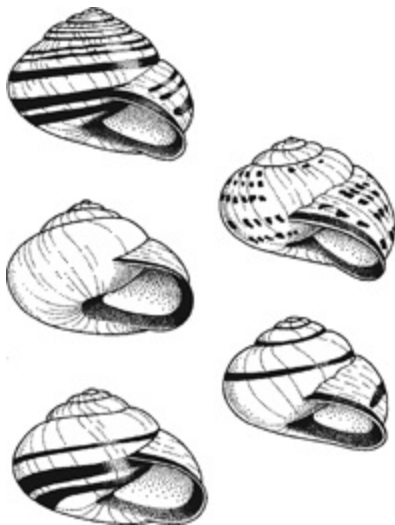
To understand the forces maintaining genetic diversity for different characters, it is crucial to clarify the selective forces acting on them. Selective values for different characters range from negligible or weakly selected to strongly selected, as illustrated in Fig. 9.4. The great majority of mutations in untranslated DNA are believed to be neutral, or nearly so (Chapter 10). Most mutations resulting in amino acid substitutions are deleterious and removed by natural selection. For those changes in amino acid sequence that persist as protein polymorphisms, some may be neutral, and some subject to balancing

selection (Brookfield & Sharp 1994; Kreitman & Akashi 1995). A very small proportion of amino acid substitutions is advantageous. Even where there is evidence for selection on protein polymorphisms, the selective forces are usually weak (see Kimura 1983; Gillespie 1991; Hey 1999).



**Fig. 9.4** Intensities of selection on different types of characters.

There is clear evidence of selection for groups of loci found in single selective units (mtDNA, inversions and clusters of loci in linkage disequilibrium such as the vertebrate MHC and self-incompatibility loci in plants).



Visual polymorphism in snails

Visual polymorphisms (e.g. for banded vs. non-banded, and yellow vs. brown snail shells, polymorphic mimics and speckled versus melanic coloration in moths) are often subject to strong selection (Endler 1986; Mousseau *et al.* 2000). Reproductive fitness is often subjected to relatively strong selection.

## Balancing selection

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Balancing selection generally impedes the loss of genetic diversity

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The three main forms of balancing natural selection (heterozygote advantage, rare allele advantage, or selection of varying direction in time and space) are considered below. Each of these actively maintains genetic diversity. We first consider their impact on very large populations and later on small populations.

### Heterozygote advantage (overdominance)

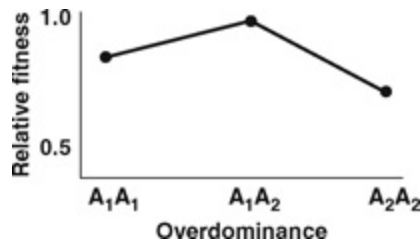
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Heterozygote advantage results in an equilibrium that actively retains polymorphism

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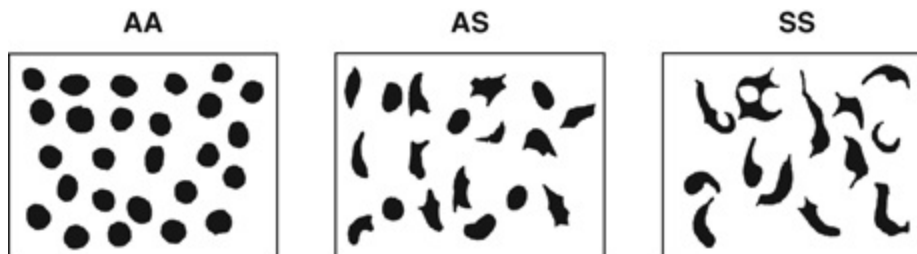
A classic example of heterozygote advantage is sickle-cell anaemia in humans living in malarial areas ([Box 9.1](#)). Heterozygotes show increased resistance to malaria, while homozygous normal individuals suffer elevated mortality from the disease. Homozygotes for the sickle allele suffer high mortality from anaemia.





### Box 9.1 Overdominant selection and balanced polymorphism for sickle-cell anaemia in humans (Allison 2004)

Sickle-cell anaemia is due to an abnormal allele of haemoglobin (S) that results in low survival of SS homozygotes due to severe anaemia. However, the S allele provides protection against malarial infection in heterozygotes. Balancing selection was first inferred from correlations between the distribution of the sickle-cell allele and malaria across Africa, the Mediterranean and Asia (see chapter frontispiece).



Blood cells of the AA, AS and SS genotypes under oxygen starvation

Allison's genotype frequency data on the haemoglobin locus for infants and adults in malarial areas of Africa are given below (after Falconer & Mackay 1996). Estimates of the relative fitnesses of the three genotypes show heterozygote advantage. The population is in Hardy–Weinberg equilibrium at birth, but selection leads to an excess of heterozygotes in adults (Problem 9.2).

	Genotype			Frequency (S)
	AA	AS	SS	
Number of infants	18	89	9	
Number of adults	400	249	5	
Frequency in infants	0.659	0.310	0.031	0.186
Frequency in adults	0.612	0.381	0.008	0.198
Relative survival				
adult frequency				
infant frequency	0.929	1.228	0.242	
Fitness relative to AS	0.929/1.228	1.228/1.228	0.242/1.228	
	0.757	1	0.197	
Selection coefficient	$s_1 = 1 - 0.76$ $= 0.24$		$s_2 = 1 - 0.20$ $= 0.80$	

The frequency of the S allele is similar in infants and adults, as expected for a population in equilibrium.

The equilibrium frequency with heterozygote advantage depends only on the relative values of the selection coefficients against the two homozygous genotypes

For a locus with two alleles subject to overdominant selection, the equilibrium frequency is obtained by equating the expression for  $\Delta q$  from [Table 6.2](#) to zero and solving, as follows:

$$\Delta q = \frac{pq (s_1 p - s_2 q)}{(1 - s_1 p^2 - s_2 q^2)} = 0$$

where  $s_1$  and  $s_2$  are the selection coefficients for  $A_1A_1$  and  $A_2A_2$ , respectively (Fig. 6.4). Equilibrium occurs when the bracketed portion of the numerator is zero, so

$$\hat{q} = \frac{s_1}{(s_1 + s_2)} \quad (9.3)$$

Thus, the equilibrium frequency depends only on the relative values of the two selection coefficients. For example, when  $s_1 = s_2$ , the equilibrium frequency is 0.5. In Example 9.2, this equation is used to predict the equilibrium frequency for sickle-cell anaemia, based on mortality data. The predicted equilibrium of 0.23 for the S allele is close to that observed in malarial areas of Africa.

This is a stable equilibrium, i.e. populations begun at the equilibrium frequency remain at that frequency, while those with frequencies above or below the equilibrium move towards it (Example 9.3).

---

### **Example 9.2 Predicted equilibrium frequency for sickle-cell anaemia, due to heterozygote advantage**

The predicted equilibrium frequency for the S allele is obtained by substituting  $s_1 = 0.24$  and  $s_2 = 0.80$  (from Box 9.1) into Equation 9.3, as follows:

$$\hat{q} = \frac{s_1}{(s_1 + s_2)} = \frac{0.24}{(0.24 + 0.80)} = 0.23$$

Consequently, the predicted equilibrium frequencies are 0.23 for the

sickle-cell allele and  $1 - 0.23 = 0.77$  for the normal haemoglobin allele.

### Example 9.3 Stable equilibrium with heterozygote advantage

To test whether the equilibrium for sickle-cell anaemia in malarial areas is stable, we can theoretically perturb its frequency to values above and below the equilibrium and determine the impacts on  $\Delta q$ . If the equilibrium is stable, then selection moves frequencies back towards the equilibrium. For example, if we perturb the frequency for S from 0.23 (equilibrium), to a value of 0.5  $\Delta q$  will be negative, while  $\Delta q$  is positive when  $q = 0.1$ .

The values of  $\Delta q$  when the frequency of the S allele is 0.5, 0.23 and 0.1 are determined below ( $s_1 = 0.24$  and  $s_2 = 0.80$ ) (Box 9.1).

For  $q = 0.5, p = 0.5$

$$\Delta q = \frac{pq (s_1p - s_2q)}{(1 - s_1p^2 - s_2q^2)} = \frac{0.5 \times 0.5 (0.24 \times 0.5 - 0.8 \times 0.5)}{[1 - (0.24 \times 0.5^2) - (0.8 \times 0.5^2)]} = -0.095$$

For  $q = 0.23, p = 0.77$

$$\Delta q = \frac{0.77 \times 0.23 (0.24 \times 0.77 - 0.8 \times 0.23)}{[1 - (0.24 \times 0.77^2) - (0.8 \times 0.23^2)]} = 0$$

For  $q = 0.1, p = 0.9$

$$\Delta q = \frac{0.9 \times 0.1 (0.24 \times 0.9 - 0.8 \times 0.1)}{[1 - (0.24 \times 0.9^2) - (0.8 \times 0.1^2)]} = 0.015$$

This equilibrium is stable, as the perturbed frequencies move back towards the equilibrium from above and below, while the population at the equilibrium frequency remains unchanged.

---

Other polymorphisms associated with heterozygote advantage for resistance to malaria include thalassaemias and the sex-linked glucose-6-phosphate dehydrogenase deficiency (Tishkoff *et al.* 2001) (see chapter frontispiece).

Some protein polymorphisms are influenced by balancing selection, based on tests of DNA sequence data. This includes the alcohol dehydrogenase locus in fruit flies (Kreitman 1983; Fig. 3.2). In addition, the warfarin resistance polymorphism in rats shows overdominance, as do most visual polymorphisms. However, several lines of evidence indicate that only a modest proportion of loci exhibit overdominance (Kimura 1983; Falconer & Mackay 1996; Nei 2005). For example, haploid organisms have similar (or higher) levels of allozyme diversity to diploids, but do not have heterozygotes. Tests across six species of native mice in North America on a total of 151 loci revealed only 11 loci deviating from neutrality, two in the direction of balancing selection and nine indicating spatially varying selection (Storz & Nachman 2003).

### **Rare allele advantage**

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A balanced polymorphism results if an allele is favoured when rare, but selected against when common

---

Rare allele advantage (also referred to as frequency-dependent selection, the Red Queen hypothesis, or the moving target hypothesis) arise under a range of realistic circumstances, especially in relation to disease (Sommer

2005). Genotypes that differ in disease resistance may be subject to rare allele advantage. Pathogens adapt to infect the more common genotypes, thereby reducing their fitness and leaving rare genotypes least affected (Lively & Dybdahl 2000). In time, rare alleles will increase in frequency and, in turn, the pathogen will adapt to them as they become common. This results in a cycling of allele frequencies and fitnesses.

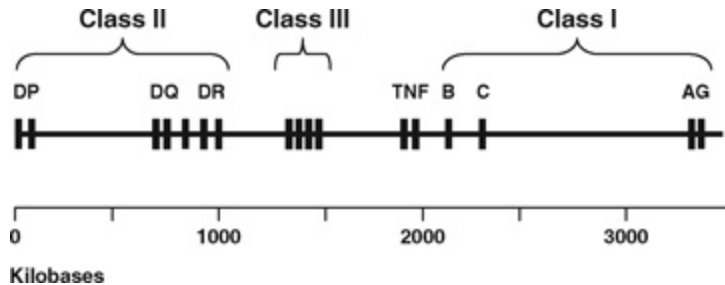
The MHC has a major role in fighting pathogens in vertebrates. There is strong evidence that balancing selection is retaining the high levels of genetic diversity at the MHC in humans and other vertebrates (Box 9.2). However it is not clear whether this selection is through heterozygote advantage or rare allele advantage, although both may be involved. High levels of genetic diversity have also been found in plant disease resistance genes that may be due to rare advantage selection (Dodds *et al.* 2006).

If genotypes use slightly different resources, one genotype may be favoured when rare, as its resource is abundant, but disadvantaged when common, as its resource is over-exploited. Predation often yields rare advantage selection on prey species displaying visual polymorphism. Birds form searching images based on common prey phenotypes, such that rarer phenotypes have greater survival. However, when the previously rare types become most plentiful, they become the basis for searching images (Clarke 1969). Rare advantage has also been reported for male colour patterns in guppy fish in the presence of predatory fish (Olendorf *et al.* 2006). In spite of these examples, selection favouring rare alleles probably maintains genetic diversity at only a small proportion of loci (Falconer & Mackay 1996).

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**Box 9.2 Balancing selection on the major histocompatibility complex (MHC) (after Hedrick & Kim 2000; Garrigan & Hedrick 2003; Sommer 2005; Piertney & Oliver 2006)**

The human MHC (called the HLA) contains over 100 loci covering a region of nearly 4 million bases of DNA. Loci fall into three main groups termed class I, II and III. Within each there are closely related loci that have arisen by gene duplication.

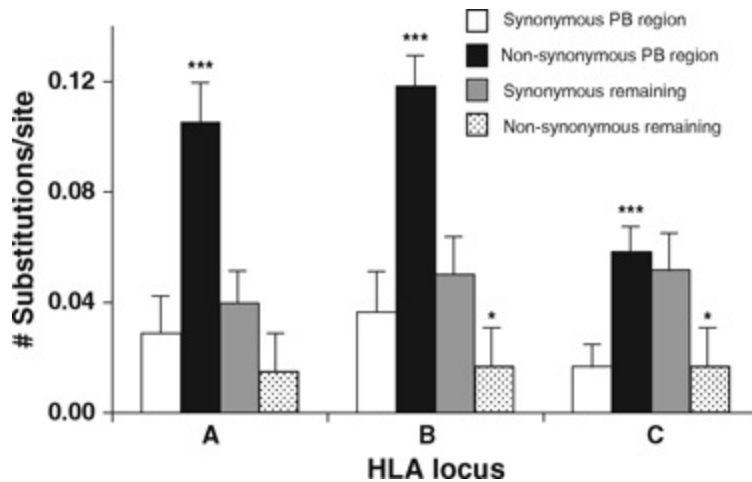


These are the major loci involved in fighting pathogens, combating cancer, and controlling transplant acceptance/rejection (see Hedrick & Kim 2000).

MHC loci exhibit the highest polymorphism of all known functional loci in vertebrates. For example, humans worldwide have 243, 499 and 321 alleles at the MHC class I HLA-A, HLA-B and HLA-C loci (Piertney & Oliver 2006). High MHC variability has also been found in many other vertebrate species (Sommer 2005).

Several lines of evidence indicate that variation at MHC loci is maintained by balancing selection:

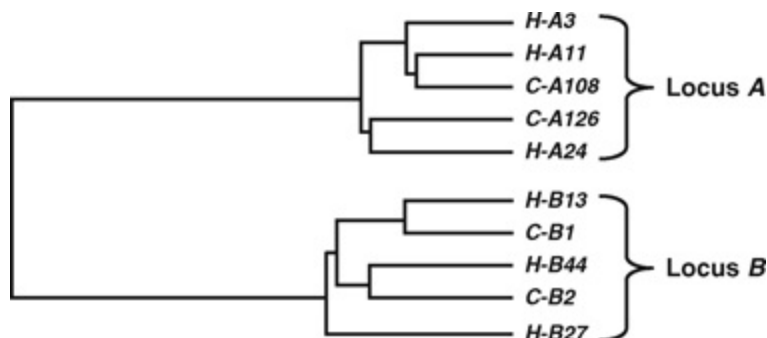
1. There are excesses of non-synonymous substitutions (causing amino acid changes) over synonymous substitutions in the functionally important peptide binding regions (PBR) of six human HLA loci, as illustrated for HLA-A, HLA-B and HLA-C below (after Hughes & Yeagher 1998). This is also evident in many other vertebrates, including bighorn sheep, wolves, Przewalski's horses, primates, giant pandas, sparrows and fish (Garrigan & Hedrick 2003; Bonneaud *et al.* 2004; Sommer 2005; Wan *et al.* 2006). By contrast, most other loci (and the non-PBR regions of these loci) show a strong excess of synonymous over non-synonymous substitutions.



\* $P < 0.05$ , \*\*\* $P < 0.001$  for comparisons of rates of non-synonymous versus synonymous substitutions.

2. Allelic frequencies at MHC loci are more even than expected for neutral alleles.

3. Polymorphisms are very ancient and extend beyond species boundaries (**trans-species polymorphism**) in many taxa, including primates, salmonids, ungulates, pinnipeds, rodents, geckos and warblers (Piertney & Oliver 2006). For example, at both the HLA-A and HLA-B loci, each chimpanzee (C) allele is more closely related to a human (H) allele than to other chimpanzee alleles, as illustrated by the gene tree below (after Nei & Hughes 1991). Polymorphisms shared by humans, chimpanzees and other primates must have been present in their common ancestor about 30 million years ago (Piertney & Oliver 2006).



4. Excesses of heterozygotes at MHC loci have been reported in aboriginal human populations, mice and pheasants, but not in bighorn



sheep (Hedrick & Kim 2000; Garrigan & Hedrick 2003).

5. There is linkage disequilibrium among MHC loci (see Box 4.4) which is probably due to selection on closely linked loci.

6. Direct associations between MHC genotypes and resistance to pathogens have been reported. For example, heterozygote advantage for particular MHC loci has been found for human responses to hepatitis B and HIV infections (Thurz *et al.* 1997; Carrington *et al.* 1999). Further, particular MHC alleles are associated with resistance/susceptibility in many vertebrates, including to HIV, malaria, hepatitis B and C, tuberculosis and leprosy in humans, to intestinal nematodes in Soay sheep, to Marek's disease tumours in chickens, to malaria in house sparrows and to bacterial and viral diseases in Atlantic salmon (Sommer 2005; Bonneaud *et al.* 2006; Piertney & Oliver 2006).

Other selective forces may also operate on the MHC. Spontaneous abortion rates are higher for couples who share MHC alleles than for those who do not (Hedrick & Kim 2000). Further, a number of mammals have shown preferences for paternity from males differing from mothers in MHC alleles. Consequently, there may be selection for avoidance of close inbreeding (Schwensow *et al.* 2008).

In spite of the strong evidence for balancing selection on the MHC, the selection coefficients are often small, being 4.2%, 1.9%, 1.5%, 0.85%, 0.28%, 0.26% and 0.07% for different loci in humans, while a selection coefficient of 0.1% was found across two species of mice (Satta *et al.* 1994; Garrigan & Hedrick 2003). Conversely, very strong selection has been reported in some cases (Black & Hedrick 1997). These differences may relate to how recently and frequently populations were subject to serious disease outbreaks. Most evidence is compatible with selection having operated at some time in the evolutionary history of the species, and not necessarily operating currently. The relative importance of the various forms of balancing selection operating on the MHC remains unclear.

The clearest case of rare advantage selection occurs at self-incompatibility (SI) loci in plants ([Box 9.3](#)). These are among the most highly polymorphic loci known, with variability akin to that of the MHC (Charlesworth & Awadalla [1998](#)). Self-incompatibility systems have important implications in conservation biology as loss of SI alleles in small populations leads to reduced reproductive fitness ([Chapter 11](#)). Alleles at the sex locus in haplo-diploids are also maintained by rare advantage selection and their loss affects population viability (Hasselmann & Beye [2004](#); Zayed & Packer [2005](#); [Chapter 17](#)).

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### **Box 9.3 Maintenance of self-incompatibility alleles in plants by rare advantage selection (after Richman & Kohn [1996](#); Castric & Vekemans [2004](#))**

Self-incompatibility ([Chapter 2](#)) has evolved as an inbreeding avoidance system in many different plant groups. It exists in several different forms. We will describe only multi-allelic gametophytic self-incompatibility due to a single locus, as found in Scrophulariaceae, Onagraceae, Papaveraceae, Solanaceae, Rosaceae and several other flowering plant families.

Gametophytic self-incompatibility has the following characteristics:

- the compatibility of matings is controlled by SI alleles at a single locus
- populations contain many SI alleles (14–193) in large populations
- if a haploid pollen grain shares an SI allele with a diploid female stigma, it will fail to fertilize the ovules (e.g.  $S_1$  pollen on a  $S_1S_3$  stigma)
- pollen from a plant genetically different from the females will produce fertile seeds (e.g. pollen from a  $S_2S_4$  plant can potentially fertilize all the ovules of a  $S_1S_3$  plant)
- trans-species polymorphisms: self-incompatibility polymorphisms are very ancient, often pre-dating speciation of related species or genera

- rare advantage selection maintains the polymorphism for self-incompatibility alleles.

An example of the operation of this system is given below for a three-allele system (after Hedrick 2005a). The table below gives the relationships between genotype frequencies in succeeding generations. Since only heterozygotes can be formed,  $P_{12} + P_{13} + P_{23} = 1$ .

Female parent	Pollen	Frequency	Offspring		
			$S_1S_2$	$S_1S_3$	$S_2S_3$
$S_1S_2$	$S_3$	$P_{12}$	—	$\frac{1}{2} P_{12}$	$\frac{1}{2} P_{12}$
$S_1S_3$	$S_2$	$P_{13}$	$\frac{1}{2} P_{13}$	—	$\frac{1}{2} P_{13}$
$S_2S_3$	$S_1$	$P_{23}$	$\frac{1}{2} P_{23}$	$\frac{1}{2} P_{23}$	—
1.0			$\frac{1}{2}(1 - P_{12})$	$\frac{1}{2}(1 - P_{13})$	$\frac{1}{2}(1 - P_{23})$

The frequencies of the three genotypes in the next generation are given at the bottom of the table. The change in genotypic frequency for  $S_1S_2$  is

$$\begin{aligned}\Delta P_{12} &= P'_{12} - P_{12} = \frac{1}{2}(1 - P_{12}) - P_{12} \\ &= \frac{1}{2}(1 - 3P_{12})\end{aligned}$$

The equilibrium genotype frequency is obtained by setting  $\Delta P_{12} = 0$ , yielding

$$\hat{P}_{12} = \frac{1}{3}$$

The equilibrium frequencies for  $P_{13}$  and  $P_{23}$  are also  $\frac{1}{3}$ , i.e. equal frequencies of all alleles. This equilibrium is reached rapidly as the selection is strong.

If the three female parents have equal frequencies, but  $S_1$ ,  $S_2$  and  $S_3$  pollen have unequal frequencies of  $\frac{1}{2}$ ,  $\frac{1}{3}$  and  $\frac{1}{6}$  (above, at and below equilibrium, respectively), then all alleles will have a frequency of  $\frac{1}{3}$  in the next generations. Consequently, pollen alleles with frequencies above the equilibrium have lower relative fitnesses, and alleles with frequencies less than the equilibrium have higher relative fitnesses, i.e. the fitnesses are dependent on allele frequencies and the equilibrium is stable.

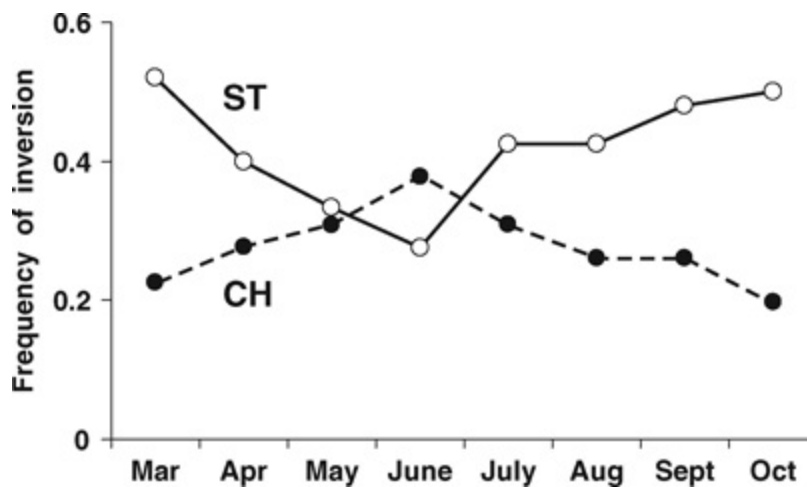
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## Selection in different directions in heterogeneous environments

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Selection that differs across seasons may lead to retention of genetic diversity

Selection that fluctuates over seasons may lead to a stable polymorphism (Haldane & Jayakar 1963). For example, the CH inversion in fruit flies in California is favoured in June and selected against in March and October and this pattern has persisted over time (Fig. 9.5).



**Fig. 9.5** Changes with season in the frequency of chromosomal inversions (CH and ST) segregating in fruit flies at Pînon Flats, California (Dobzhansky *et al.* 1977). This polymorphism showed a similar pattern in different years, confirming that it is stable.

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When there is differential selection in diverse habitats and migration among them, a polymorphism may result

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[Box 9.4](#) details a cline in glaucousness (leaf waxiness) with elevation in several species of eucalypt trees in Tasmania, Australia, which is maintained by a balance between migration and selection. Selection for frost tolerance favours glaucous individuals at higher elevation, while selection due to insect defoliation acts against them at lower elevation. Clines due to migration–selection balance have been found for heavy-metal tolerance in colonial bent grass plants between old heavy-metal mine waste sites and nearby pastures in Wales ([Chapter 7](#)), for industrial melanism in peppered moths across gradients from polluted to unpolluted areas (Bishop & Cook [1975](#)) and for alleles at several allozyme loci (Powers *et al.* [1991](#)). Clines in morphological characters are relatively common, some being so pervasive that they are referred to as ecogeographic rules ([Chapter 7](#)). However, conditions for maintenance of genetic diversity by mechanisms involving spatial or temporal variation in selection are considered to be rather restricted (Prout [2000](#)). Nevertheless, tests on allozyme polymorphisms in native mice indicated that diversifying selection was more common than balancing selection (Storz & Nachman [2003](#)).

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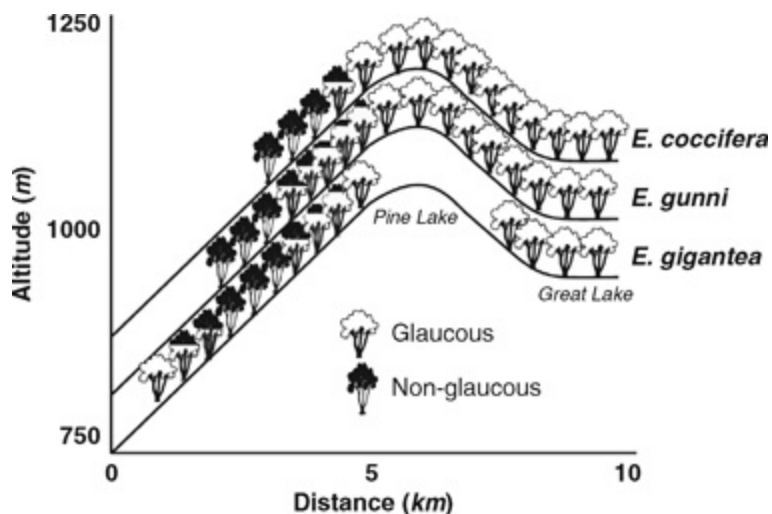
**Box 9.4 Clines in leaf glaucousness in several species of eucalypt trees in Tasmania, Australia due to differential selection different altitudes balanced by pollen flow (Barber [1955](#); Barber & Jackson [1957](#); Thomas & Barber [1974](#))**

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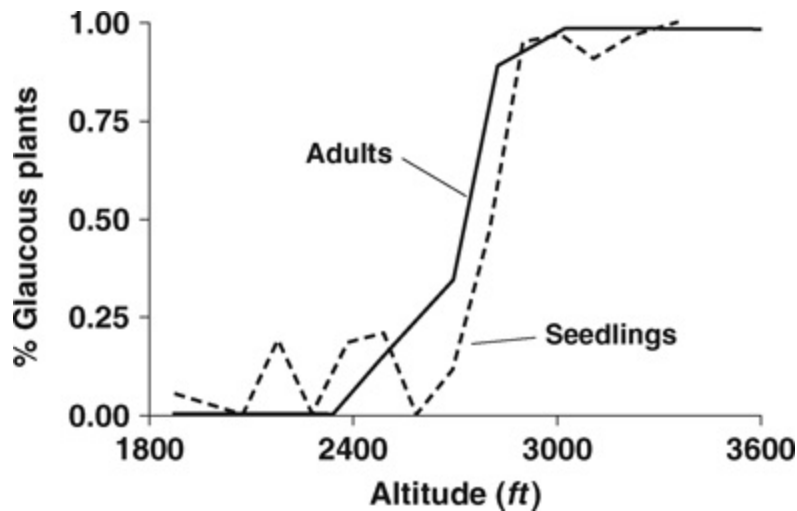
Some eucalypt trees have leaves with a distinct waxy (glaucous) layer on them. At least eight species of these gum trees in Tasmania have parallel clines of glaucousness, with greater frequencies of waxy leaves at higher, frosty, altitudes. Further, some species show similar clines in different locations.



Eucalypt trees



Glaucous leaves have greater survival in heavy frosts. At lower elevations, glaucous plants suffer greater defoliation through insect attacks. Selective differences have been demonstrated by showing that frequencies differ between seedlings and adult plants (see lower figure).



Pollen flow among populations results in mixing of alleles from different elevations, while selection operating between seedling and adult stages of the life cycle re-establishes differences among elevations, as shown for the urn gum above (after Barber [1955](#)).

## Reproductive fitness

Mutation–selection balance is widely acknowledged as an important factor maintaining genetic diversity for reproductive fitness

Genetic diversity for loci affecting reproductive fitness can potentially be maintained by the processes mentioned above, with the exception of neutral mutations since these, by definition, do not influence fitness.

A substantial proportion of genetic diversity for fitness characters is due to mutation–selection balance, but the precise proportion is unclear (Charlesworth & Hughes 2000; Rodríguez-Ramilo *et al.* 2004). Neither overdominance nor rare advantage selection are currently considered to be important means for maintaining quantitative genetic diversity for fitness, but there may be some contribution from selection that varies in space or time (Charlesworth & Hughes 2000; Grant & Grant 2002).

## Maintenance of genetic diversity in small populations

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Genetic drift has a larger impact, and balancing selection is less effective, in smaller populations

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Genetic diversity is generally lower in small populations than in large populations (Chapter 3). This arises because genetic drift has a larger impact in small populations and balancing selection is less effective. Five crucial points emerge:

- drift fixes alleles more rapidly in smaller populations
- loci subject to weak selection in large populations approach effective neutrality in small populations
- mutation–selection equilibria are lower in small than in large populations
- the effect of finite population size on balanced polymorphisms depends on the equilibrium frequency. The fixation of intermediate frequency alleles is retarded by selection, but this mechanism accelerates fixation of low frequency alleles
- balancing selection can retard loss of genetic diversity, but does not



prevent loss in small populations.

## Selection and drift in small populations

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Genetic drift has a major impact in small populations even for loci that are subject to balancing selection.

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The balance between selection and drift depends on the population size and the intensity of the selection (Willi *et al.* [2006](#)). When both factors are operating, selection predominates in very large populations, while drift predominates in small populations, as we saw for the red flour beetle data in [Fig. 8.2](#).

## Drift may negate the influence of selection

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An allele is effectively neutral if its selection coefficient is less than  $\sim 1/(2N_e)$

---

In small populations, alleles that do have effects on fitness may behave as if they are not subject to selection, and drift randomly in frequency from one generation to the next (Wright [1931](#)). Weakly selected alleles in small populations, plus strictly neutral alleles, are referred to as **effectively neutral** (or selectively neutral).

The conditions for effective neutrality depend on the relationship between the selection coefficient and the effective population size. The distributions of allele frequencies for weakly selected loci are very similar to those for neutrality ( $s = 0$ ) until  $s > 1/(2N_e)$ . Consequently, Kimura (1983) defined an effectively neutral allele as one where

$$s < \frac{1}{2N_e} \quad (9.4)$$

For example, a selection coefficient of 5% (a very strong deterministic force in a large population) becomes effectively zero in a population of effective size  $< 10$ . Further examples of population sizes required for alleles to be effectively neutral are given in Example 9.4. These indicate that most allozyme loci will be effectively neutral in populations with effective sizes of less than 300. MHC alleles may often behave as if neutral in populations with effective sizes of 50 or less. As effective population sizes may be about 10% of actual sizes (Chapter 11), allozyme and DNA polymorphisms will often behave as though neutral, or very nearly so, for most populations of conservation concern.

---

#### **Example 9.4 In what sized populations are alleles effectively neutral?**

Allozymes with selection coefficients of 0.15% or less (Kreitman 1996) will be effectively neutral when  $s < 1/(2N_e)$ , thus

$$0.0015 < \frac{1}{2N_e}$$

i.e. when

$$N_e < \frac{1}{2 \times 0.0015} < 333$$

Thus, allozyme alleles will be effectively neutral in populations with effective sizes of about 300 or less.

A MHC allele with a 1% selection coefficient will be effectively neutral when

$$0.01 < \frac{1}{2N_e}$$

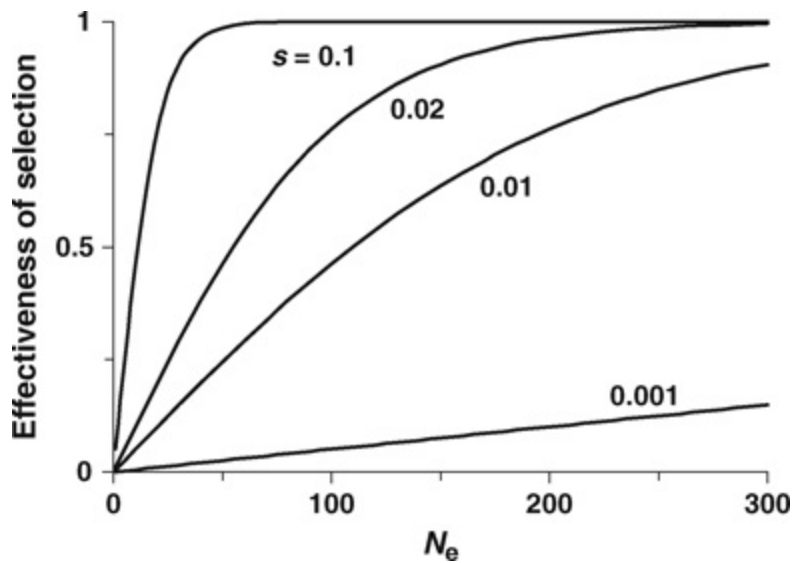
i.e. when

$$N_e < \frac{1}{2 \times 0.01} < 50$$

Thus, an allele with a selection coefficient of 1% will be effectively neutral in a population with an  $N_e$  of less than 50.

---

While [Equation 9.4](#) suggests that there is a threshold population size below which effective neutrality occurs, the effectiveness of selection actually declines in a more-or-less continuous fashion as effective population size is reduced ([Fig. 9.6](#)). An allele with a selection coefficient of 10% is selected as effectively as in an infinite population size until  $N_e$  drops below 50. Below this, the effectiveness of selection drops rapidly as  $N_e$  reduces and the allele becomes effectively neutral. An allele with a selection coefficient of 1% is not selected with complete efficiency even in a population of 300.



**Fig. 9.6** Selection is less effective in small than in large populations. Effectiveness of selection is plotted against  $N_e$  for alleles with different selection coefficients ( $s$ ). An allele with an effectiveness of 1 has the same probability of fixation as that in an infinite population. Effectiveness is defined as  $(P_{\text{fixation}} - p)/(1 - p)$ , where  $p$  is the initial frequency of the allele and  $P_{\text{fixation}}$  is the probability of fixation for the allele in populations of particular sizes.

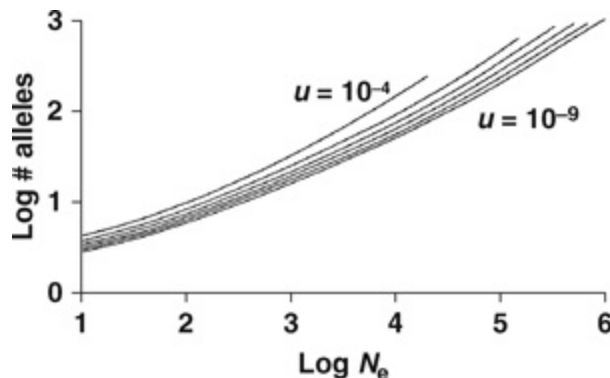
## Balancing selection and drift in small populations

Even strongly selected balanced polymorphisms for the MHC, inversions, self-incompatibility alleles and visual polymorphism lose genetic diversity due to genetic drift in small populations

Balancing selection may slow the loss of genetic diversity in small populations, but cannot normally prevent it. Whilst genetic diversity at the MHC is high in large populations due to balancing selection, it is low in many bottlenecked populations, including those of Australian bush rats,

endangered Chatham Island black robins in New Zealand and an island population of bighorn sheep, but not in island foxes (Seddon & Baverstock 1999; Aguilar *et al.* 2004; Sommer 2005). Further, both visual polymorphisms and inversions are subject to genetic drift in small populations (Lamotte 1959; Montgomery *et al.* 2000).

The number of self-incompatibility alleles is expected to be related to population size (Fig. 9.7). Losses have been documented in several small plant populations despite rare advantage selection (Les *et al.* 1991; Demauro 1993; Young *et al.* 2000).



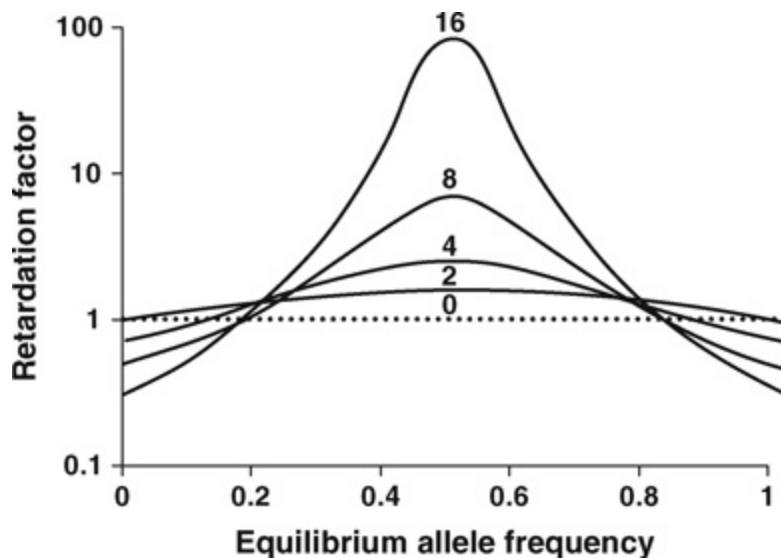
**Fig. 9.7** Smaller plant populations are expected to have fewer self-incompatibility alleles than larger populations. Predicted number of S alleles in populations with different effective sizes ( $N_e$ ) for various mutation rates ( $u$ ) (after Richman & Kohn 1996). The model involves drift, mutation and self-incompatibility selection.

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Heterozygote advantage impedes fixation for alleles with equilibrium frequencies in the range 0.2–0.8, but accelerates fixation for alleles outside this range when compared to neutral alleles

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Heterozygote advantage in small populations impedes fixation for alleles with equilibrium frequencies in the range 0.2–0.8. However, it is not widely appreciated that it actually *increases* the rate of fixation for alleles with equilibrium frequencies outside this range, compared to neutral alleles (Fig. 9.8). This occurs because alleles that drift to more intermediate frequencies are moved back towards their more extreme equilibrium frequencies by selection, thus making them more susceptible to loss by drift. In other words, selection ‘discourages’ these rarer alleles from drifting to higher frequencies. Many alleles at polymorphic DNA and allozyme loci fall into this low frequency range. Even for alleles with equilibria of 0.2–0.8, there are genetic drift effects unless selection coefficients are large and/or population sizes very large (Robertson 1962). DNA sequence polymorphisms, allozymes and even MHC diversity are likely to fall within this range of selective values in threatened populations.



**Fig. 9.8** Retardation in fixation probability in finite populations compared to that for a neutral locus, for overdominant loci with different equilibrium frequencies (after Robertson 1962). When the retardation factor exceeds 1.0, selected loci show greater retention of genetic diversity than neutral loci, but when it is less than 1.0 they show accelerated fixation. The numbers on the curves represent different values of the product of selection coefficients and effective population size [ $N_e(s_1 + s_2)$ ], with 0 being the neutral case.

*Overdominant selection retards fixation in the range 0.2–0.8, but accelerates it outside this range of equilibrium frequencies.*

The impact of natural selection on almost all endangered species has been substantially reduced since their numbers are depleted. The conservation implications are clear and extremely important.

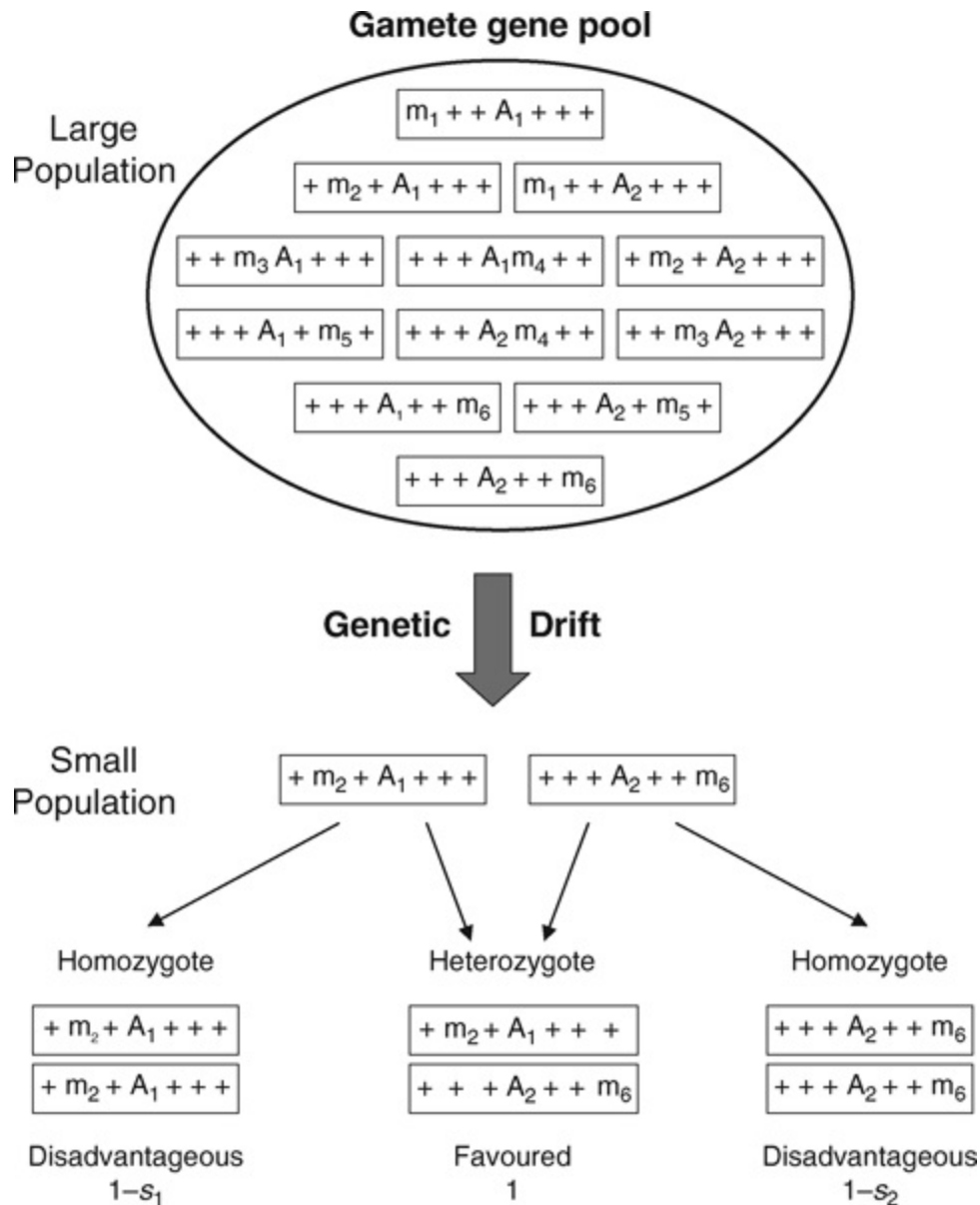
## Associative overdominance

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Heterozygote advantage for blocks of genes develops over generations in small populations, due to linkage disequilibrium between deleterious alleles (associative overdominance)

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In small populations, a further interaction between balancing selection and drift arises. **Associative overdominance** is an apparent overdominance due to linkage disequilibrium between deleterious alleles at different loci. It develops over generations in small populations, as chromosomal haplotypes are lost by chance (Fig. 9.9). It does not impede the initial loss of genetic diversity (e.g. there has been fixation at loci  $m_1$ ,  $m_3$ ,  $m_4$  and  $m_5$ ). In the latter stage, chromosomal homozygotes are homozygous for one or more deleterious recessive alleles. Conversely, chromosomal heterozygotes are heterozygous for these alleles. Consequently, chromosomal homozygotes are impaired, while heterozygotes have higher reproductive fitness. When this occurs, the fate of an allele is determined by the loci around it. The neutral locus (A) in the figure is exhibiting apparent overdominance, as it is non-randomly associated with the deleterious alleles  $m_2$  and  $m_6$ .



**Fig. 9.9** Development of associative overdominance in small populations. The gene pool for a large population is shown with an array of chromosomes that exhibit linkage equilibrium between deleterious alleles ( $m$ ) and a neutral marker locus ( $A$ ). In a small population, genetic drift over generations leads to the loss of all except two chromosomal haplotypes, leading to linkage disequilibrium (recombination is insufficient to prevent this in small populations). The resulting genotypes exhibit overdominance, as each chromosomal homozygote is homozygous for a different recessive deleterious allele ( $m_2$  or  $m_6$ ), while the chromosomal heterozygotes are heterozygous for both deleterious alleles. Consequently, neutral alleles ( $A_1$



vs.  $A_2$ ) on the chromosomes behave as if they exhibit heterozygote advantage (associative overdominance).

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Associative overdominance slows the subsequent loss of genetic diversity, but does not prevent it

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Computer simulations of small populations with many loci indicate that linkage disequilibrium between neutral loci and either deleterious alleles, or loci showing overdominance, slows fixation at neutral loci, but does not prevent eventual fixation (Latter [1998](#)).

The selective forces involved here are likely to be much stronger than those experienced by most single loci, i.e. the major selective force in small populations may usually be associative overdominance.

As the behaviour of individual loci is often affected by nearby loci, [Chapter 10](#) is concerned with the evolutionary genetics of whole genomes (**population genomics**). Comparisons between the behaviour of many loci typically have much greater power for detecting selection than analyses on individual loci.

## Summary

1. Neutral mutation, random genetic drift and balancing selection are the major mechanisms responsible for genetic diversity due to intermediate frequency alleles in natural populations.

2. Balancing selection usually impedes loss of genetic diversity. This may take the form of heterozygote advantage, rare advantage selection, or selection of varying direction over space or time.
3. The relative contributions of selection versus drift in determining levels of genetic diversity depend on the population size and the character being considered, with drift effects predominating in small populations, while selection is most effective in large populations.
4. Selection is more important for visual morphological polymorphisms and reproductive fitness than for untranslated DNA, with protein polymorphisms intermediate. Drift has the opposite pattern of importance.
5. Genetic variation for reproductive fitness is maintained due to a combination of mutation–selection balance and balancing selection.
6. Population size is a major determinant of genetic diversity for all loci and characters in small populations and species of conservation concern.

### **Further reading**

Allison ([2004](#)) Excellent historical review on heterozygote advantage for sickle-cell disease in malarial areas in Africa.

Castric & Vekemans ([2004](#)) Wide-ranging review of the evolutionary biology of self-incompatibility in plants.

Hedrick ([2005a](#)) *Genetics of Populations*. Thorough coverage of topics in this chapter.

Kimura ([1983](#)) *The Neutral Theory of Molecular Evolution*. A fine exposition of the neutral theory for maintenance of genetic diversity.

Nei ([2005](#)) Wide-ranging review of molecular evolution, morphological evolution and maintenance of genetic variation.

Piertney & Oliver ([2006](#)) Review of mechanisms maintaining genetic diversity at MHC loci in a broad range of vertebrates.

### **Software**

DNAsp: Software to carry out several statistical tests of neutrality.  
[www.uc.es/dnasp/](http://www.uc.es/dnasp/)

EASYPOP: Software for simulating the effects of mutation, population size, migration and population fragmentation (Balloux 2001).  
[www.unil.ch/dee/page36926\\_fr.html](http://www.unil.ch/dee/page36926_fr.html)

SELECTION: Software to simulate the effects of selection, drift, mutation and migration on a single locus. [www.gsoftnet.us/GSoft.html](http://www.gsoftnet.us/GSoft.html)

WINPOP 2.5: Software to simulate selection, drift, gene flow and migration. [www.genedrift.org/winpop.php/](http://www.genedrift.org/winpop.php/)

## Problems

- 9.1 Genetic diversity under neutrality. What are the predicted equilibrium heterozygosities and effective number of alleles under neutrality for a locus with a neutral mutation rate of  $10^{-7}$  in a population with an effective size of 20?
- 9.2 Excess heterozygosity due to selection. Do the genotype frequencies at the haemoglobin locus for adults differ from Hardy–Weinberg equilibrium expectations for the data in Box 9.1? Do the infant genotype frequencies differ from Hardy–Weinberg equilibrium expectations?
- 9.3 Heterozygote advantage. If relative fitnesses are 0.99, 1 and 0.97 for genotypes  $A_1A_1$ ,  $A_1A_2$  and  $A_2A_2$ , respectively, what are equilibrium frequencies for the two alleles?
- 9.4 Heterozygote advantage. What is the equilibrium frequency for the warfarin resistant R allele in rats in the presence of warfarin poison, given the following survival rates of the three genotypes (modified from Greaves *et al.* 1977)? (Assume that selection is solely on survival)

RR	RS	SS
0.3	0.8	0.56

- 9.5** Heterozygote advantage. If the relative fitnesses of the three genotypes  $A_1A_1$ ,  $A_1A_2$  and  $A_2A_2$  at a locus are 0.7, 1 and 0.9, what will be the final state of populations beginning with a frequency of  $A_1$  of (a) 0.1? (b) 0.3? (c) 0.9?
- 9.6** Equilibrium frequency with heterozygote advantage. Derive the expression for the equilibrium frequency due to selection favouring heterozygotes at a locus. Assume that the starting frequencies for alleles  $A_1$  and  $A_2$  are  $p$  and  $q$ .

	Genotypes			Total
	$A_1A_1$	$A_1A_2$	$A_2A_2$	
Frequencies at fertilization				
Relative fitnesses	$1 - s_1$	1	$1 - s_2$	
After selection				
Adjust so total is 1				
New frequency of $A_1 = p_1 =$				
$\Delta p =$				
At equilibrium $\Delta p =$				

- 9.7** Self-incompatibility. For the system described in [Box 9.3](#), determine the relative fitness of each SI allele in pollen in a population with equal frequencies of three genotypes in females, but frequencies of  $S_1$ ,  $S_2$  and  $S_3$  alleles of  $1/6$ ,  $1/3$  and  $1/2$  in pollen.
- 9.8** Self-incompatibility. What will the relative fitness of a new  $S_4$  allele be in pollen in the case described in [Box 9.3](#), if the three female genotypes shown have equal frequencies and alleles  $S_1$ ,  $S_2$ ,  $S_3$  and  $S_4$  have frequencies of 0.33, 0.33, 0.33 and 0.01, respectively, in pollen?
- 9.9** Selective neutrality. At what population size is an allele with a selection coefficient of 2% effectively neutral?

## Practical exercises: Computer simulations

Use SELECTION or a similar software package to simulate aspects of the

maintenance of genetic diversity due to heterozygote advantage in large vs. small populations.

### 1. Strong selection

Simulate the allele frequency trajectories for sickle-cell anaemia, beginning at different allele frequencies, using the relative fitnesses observed by Allison in 1956:

AA	AS	SS
0.76	1	0.20

Commence runs with S allele frequency 0.1 and run for 100 generations with (i) an infinite (or very large) population, (ii)  $N = 100$  and (iii)  $N = 10$ , doing 20 replicates of each of the latter two. Compare the outcomes for the three population sizes.

### 2. Weak selection: equilibrium $q = 0.5$

Simulate the allele frequency changes for the following model of heterozygote advantage with weak selection:

$A_1A_1$	$A_1A_2$	$A_2A_2$
0.99	1	0.99

Commence runs with  $q = 0.5$  and run for 100 generations (i) an infinite population, (ii)  $N = 100$  and (iii)  $N = 10$ . Run 50 replicates of the latter two cases. Repeat the runs for same population sizes with neutrality (relative fitnesses of all genotypes of 1). Compare the proportion of populations polymorphic at generation 100 for the neutral cases with those for balancing selection and across population sizes. Does balancing selection slow fixation, or speed it up?

### 3. Weak selection: equilibrium $q = 0.1$

Simulate the allele frequency changes for the following model of heterozygote advantage with weak selection:

$A_1A_1$	$A_1A_2$	$A_2A_2$
0.99	1	0.999

Commence runs with  $q = 0.1$  and run for 100 generations with (i) an infinite population, (ii)  $N = 100$  and (iii)  $N = 10$ . Run 50 replicates. Repeat the runs for the same population sizes with neutrality. Compare the proportion of populations polymorphic at generation 100 for the neutral cases with those for balancing selection, and across population sizes. Does balancing selection slow fixation, or speed it up?

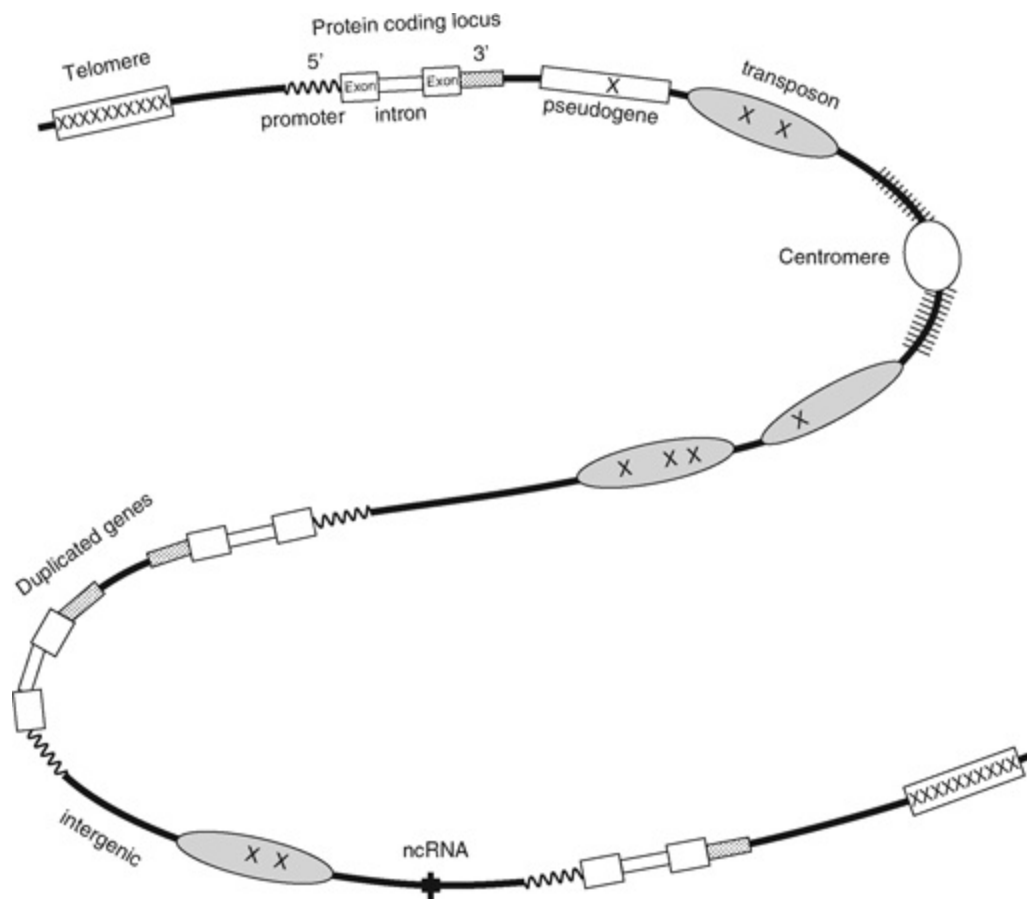
## Chapter 10 Population genomics

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Improved insights into genome structure and the evolutionary forces operating on genomes have come from whole genome sequences and from multilocus gene expression studies. Only a small proportion of the genome codes for proteins and there are additional non-translated functional sequences. Large proportions of eukaryotic genomes have no known function, and contain many transposons. Whole genome analyses allow powerful tests to differentiate loci subject to selection from those exhibiting genetic drift and to identify the mode of selection. The tools and insights of genomics may, in time, lead to genetic management practices to augment current, whole genome management with individual management for loci of particular adaptive importance

### Terms

Background selection, cDNA, cis, genome, genome enabled taxa, indel, microarray, miRNA, non-coding RNA (ncRNA), null allele, population genomics, purifying selection, RNAi, selective sweep, siRNA, transcriptome, untranscribed region (UTR)



Representation of portion of a genome (a chromosome) with telomeres, protein coding locus (including promoter, exons, introns, termination signals and 3' untranscribed regions), intergenic regions, transposons, pseudogene, centromere, duplicated loci and ncRNA

## Genome sequencing and population genomics



Population genomics is the study of evolutionary processes for entire genomes

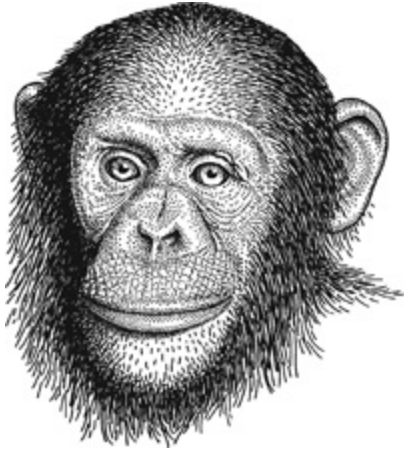
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Up to this point we have dealt predominantly with individual loci in isolation from others. The study of entire genomes (**genomics**) provides enhanced insights into the function, structure and evolution of whole organisms. It provides powerful tools for analyses within and among populations and species.

As of November 2007, 730 species had been sequenced, including 125 eukaryotes, 557 bacteria and 48 archaea. Genome sequencing projects are under way or planned for many additional species. Of the sequenced eukaryotes, 17 are vertebrates and 41 higher plants, along with invertebrates and lower eukaryotes ([Box 10.1](#)). They include humans, chimpanzees, African elephants, rats, mice, 12 different species of fruit flies and the *Arabidopsis* plant.

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**Box 10.1 Examples of species whose genomes have been sequenced and approximate numbers of protein coding loci in each** (<http://www.ensembl.org> 2007; [http://www.genome.jp/kegg/catalog/org\\_list.html](http://www.genome.jp/kegg/catalog/org_list.html) 2007)



Chimpanzee



Fruit fly

Species	Number of protein coding loci
Mammals	
Human	23 700
Chimpanzee	20 600
Rhesus monkey	21 900
Mouse	23 800
Rat	23 000
Cat	14 800 <sup>a</sup>
Cow	21 800
African elephant	15 717 <sup>a</sup>
Opossum	19 400
Platypus	17 956
Birds	
Domestic chicken	16 700
Amphibian	
Western clawed frog	18 000
Fish (4 species)	
Pufferfish	21 900
Zebrafish	21 300
Invertebrates	
Fruit fly	14 000
Nematode worm	20 100
Plants (41 species)	
<i>Arabidopsis</i>	27 000
Poplar tree	45 600
Rice	26 841
Unicellular eukaryote	
Yeast	7 000
Bacteria (557 species)	
<i>Escherichia coli</i>	4 300

<sup>a</sup> Underestimate due to low sequence redundancy.

Few threatened species, apart from chimpanzees and African elephants, have so far been fully sequenced. However, about 1000 threatened species lie within the same Order as species that have been sequenced. As the sequenced genome of a related species can aid genomic analyses of a threatened species, the latter are referred to as **genome-enabled taxa** (Kohn *et al.* 2006). A growing number of extinct species have also been sequenced, including mammoths, extinct cave bears and Neanderthals (Noonan *et al.* 2006; Poinar

*et al.* 2006).

Genomes of mammals are very large. For example, the haploid human genome is about  $3.2 \times 10^9$  base pairs in length and other mammal genomes are of similar sizes. The typical process required to sequence a genome is to break it into fragments, clone the fragments into microbial vectors, amplify the fragments, re-extract them and run them in DNA sequencing machines to reveal the base sequences. The sequences of the different fragments must be connected (aligned) by identifying overlapping fragments (shotgun sequencing), or mapped onto chromosomes. A variety of methods are used and the technology is evolving rapidly. For example, the protein coding regions of the Glanville fritillary butterfly have been sequenced by reverse transcribing mRNA from multiple individuals into DNA (**cDNA**), followed by direct sequencing using 454 pyrosequencing (Vera *et al.* 2008).

While the cost of sequencing whole genomes is currently prohibitive for most threatened species, it is reducing rapidly. The first human genome sequence was completed in 2003 at a cost of \$US2.7 billion and 13 years, while James Watson's genome was sequenced in 2008 in 4.5 months at a cost of less than \$US1.5 million (Wadman 2008). Craig Venter has predicted that the cost of a full genome sequence will drop to \$US1000 within a decade (Jones 2006). It may not be long before whole genome sequencing for threatened species is both feasible and affordable.

However, **population genomics** depends upon both sequencing genomes and identifying polymorphic sequences within species. Identifying polymorphisms may be done directly by sequencing multiple individuals, or by indirect methods. Such studies have revealed extensive genetic diversity. Comparison of Craig Venter's own diploid genome sequence with that of the human sequence in the public database revealed over 3 million SNPs, ~ 292 000 heterozygous base insertion/deletion (**indels**) events, 90 inversions, duplications and copy number variant regions (Levy *et al.* 2007). Further, SNP variation at over 500 000 sites has been documented for > 400 humans from 29 populations across the globe (Jakobsson *et al.* 2008). To catalogue

human variation, plans have been announced to sequence 1000 humans in the next three years (Kaiser [2008](#)).

Much of the study of genomic variation focuses on SNPs. Black & Vontas ([2007](#)) and Kim & Misra ([2007](#)) review the many methods for detecting SNP variation within populations. We only have space to mention a few here. First, SNP heterozygotes may be detected following sequencing of diploid individuals, as they reveal two bases at particular sites (Stephens *et al.* [2006](#)). Second, indirect methods are used for identifying sequence polymorphism and many can be done on **microarrays** (Gilchrist & Haughn [2005](#)). Microarrays are thousands of 100–250- $\mu$ m spots of single-stranded DNA bound to microscope slides in a precise, known pattern. Robert Wayne's group at UCLA is genotyping wolves for 127 000 SNPs using a microarray chip (M. Gray and R. K. Wayne pers. comm.). Indirect methods involve two steps, allele discrimination and allele detection. Allele discrimination methods include (a) allele-specific complementary base pairing, (b) PCR amplification of specific alleles and (c) single base extension (replication, or not) across SNP sites. Allele-specific products are typically detected by mass spectrometry, fluorescence or chemiluminescence.

This chapter describes insights into genome evolution and function that have been revealed from sequencing genomes, characterizing DNA sequence variation and measuring expression of functional loci within those genomes. These provide important information for issues considered in the following chapters and augment issues considered in [Chapters 3–9](#). In particular, we discuss information that can be gained by analyses involving many loci compared to those for single loci alone and the multilocus evolutionary processes that they reveal. Further, we assess what conservation benefits might be gained from population genomics.

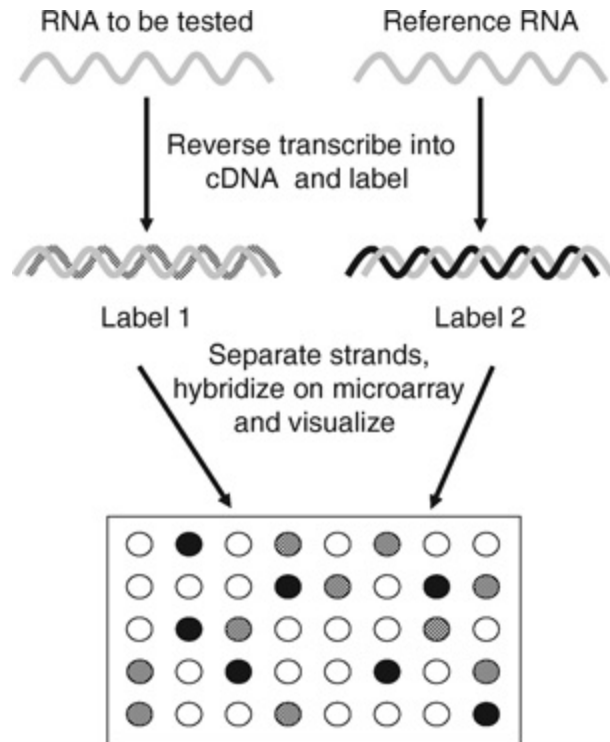
## **cDNA expression microarrays**

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Microarrays can be used to simultaneously measure transcription levels at thousands of loci in genomes

Once a species has been sequenced, microarray chips carrying many single-stranded cDNA sequences from coding regions can be manufactured and used to measure gene function (**functional genomics**) simultaneously at thousands of loci across the genome (Whitehead & Crawford 2006a). These are referred to as **cDNA expression microarrays** (Fig. 10.1). mRNA is obtained from the individuals of genotypes whose gene expression is to be measured, reverse transcribed into cDNA, fluorescently labelled and separated into single strands, prior to DNA–DNA hybridization to the immobilized single-stranded cDNA on the slide (Ranz & Machado 2006). The level of fluorescence for a particular locus is then a measure of transcription at that locus.



**Fig. 10.1** cDNA expression microarray.

Differences in levels of transcription among alleles or populations indicate possible quantitative differences of enzymes or proteins. The expression pattern of all transcribed elements (mRNAs, rRNAs, tRNAs and regulatory non-coding RNAs) in the genome is referred to as the **transcriptome**. Microarray data do not encompass all components of gene regulation, as they exclude effects at the level of mRNA splicing and at the level of the proteins.

Careful experimental controls and extensive replications are necessary to detect true genotypic differences, since transcription levels are sensitive to environmental influences and often differ among tissues, ages and sexes (Eisenstein [2006](#)). Currently, 1.5-fold or greater differences in transcription levels are being detected as statistically significant.

## **What conservation benefits might be gained from genomics?**

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Genomics will enable much more precise delineation of the genetic characteristics of populations and greater understanding of evolutionary processes, and will in some cases lead to individual-locus genetic management in threatened species

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We are not aware of any current direct use of genomic information in the genetic management of threatened species. Consequently, we will only discuss the future potential of this information and the pitfalls that may await us in applying it. Ryder ([2005](#)) and Kohn *et al.* ([2006](#)) have considered the potential benefits to be derived from conservation genomics. Whole genome sequencing and microarray analyses will provide more precise and unbiased measures of effective population size, demographic history, levels of

inbreeding, rates of gene flow, differentiation among populations and taxonomic status (Luikart *et al.* 2003). To these, we can add:

- identifying loci involved in inherited diseases in threatened species, thus allowing tests for carriers to be devised (Chapter 19)
- identifying loci subject to selection and delineating the forms of selection (relevant to Chapter 9)
- individual genetic management of loci with variation important to fitness, e.g. MHC variation for resistance to a tumour disease in Tasmanian devils (Box 10.3)
- detecting deleterious alleles responsible for inbreeding depression (Chapter 13)
- revealing the loci responsible for reproductive isolation among populations and species (Chapter 16)
- obtaining information on species biology that is important to conservation, e.g. reconstructing pedigrees and revealing demographic histories (Chapter 21)
- determining relationships among founders of captive populations (Chapter 19)
- estimating the total number of bases that can produce deleterious mutations (the target size for mutations) from the number and size of functional regions in a species (Chapters 7 and 13)
- obtaining more precise estimates of total mutation rates (Haag-Luitard *et al.* 2007; Chapters 7 and 12)
- determining the proportions of loci that exist as single versus multiple copies per haploid genome, as this influences the chance of expressing deleterious alleles (Chapter 13)
- predicting evolutionary potential of a population from polymorphism for selectively important loci (Chapter 11)
- estimating the number of loci affecting important quantitative characters, especially reproductive fitness (Chapter 5)
- obtaining insights into disease epidemiology, including the genetic basis of host shifts and changes in virulence (Chapter 21).

One of the earliest uses is likely to be identifying loci involved in inherited



diseases in threatened species from information on homologous conditions in sequenced species, e.g. identifying the chondrodystrophy locus in California condors from information on the DNA sequence of related dwarfisms in chickens. This will allow a test for carriers to be devised, so that the lethal allele can be removed from the population ([Chapter 19](#)).

Below we describe relevant findings on the nature of genomes and insights into evolution from genomics that provide important background information for conservation genetics.

## Genome organization

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The genome comprises many functional elements beside protein coding loci, including rRNA, tRNA and ncRNA loci, regulatory sequences, centromeres and telomeres. The most abundant components of most eukaryote genomes consist of predominantly non-functional intergenic regions, repetitive sequences, pseudogenes and especially transposons

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There are many components to the genome ([Table 10.1](#)). The protein coding loci represent the major functional component of the genome. However, they represent only a small proportion of the total genome. Further, many of the other components of the genome perform important functions, as detailed below. The largest component of the genome is due to transposons. These DNA parasites can create mutations by moving and inserting into loci, or by recombining and yielding duplications and deficiencies.

**Table 10.1** *Components of the genome, their approximate number of copies and the proportions they represent in humans (where known)*

Components	Function	Number (K = 1,000)	% in humans
Protein coding loci	Code for enzymes and proteins	20–25 K	1.2
Exons	Transcribed and translated	200 K	1.1
Introns	Transcribed and excised	160 K	24.0
Promoters	Control transcription	27–33 K	
3' UTR	Transcription termination	≥ 20–25 K	
Functional RNA molecules			
rRNA loci	Translation machinery	300–400 in 5 clusters	
tRNA loci	Involved in translation	~ 500	
ncRNAs	Some are regulatory molecules		
Centromeres	Critical to chromosome separations in meiosis and mitosis	46	
Telomeres	Stabilize chromosome ends	92	
Intergenic regions	Largely non-functional		27
Pseudogenes	Non-functional remnants of coding loci	20 K	
Transposons	Mobile DNA parasites	2.3 million	48

## What proportion of the genome is functional?

A very small proportion of the genome codes for proteins and an additional small proportion has regulatory and other functions

One of the surprising features of the human genome is that only 1.2% of it codes for proteins (Anonymous 2007). In addition, around 3.8% of the non-coding region is conserved compared to the mouse and presumably has important functions. Some of this will be regulatory sequences that modulate gene expression, but the functions of the remainder are unclear. The situation is likely to be similar for all mammals and relatively similar for other vertebrates, invertebrates and plants. Thus, the proportion of the genome that can produce deleterious mutations is much less than total genome sizes.

## Number of coding loci in different taxa

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Mammals contain ~ 20 000–25 000 protein coding loci, invertebrates ~ 15 000, while plants have diverse numbers, generally greater than 10 000

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Genome sequencing has allowed us to determine the number of protein coding loci in different taxa ([Box 10.1](#)). Humans have only about 20 000 to 25 000 loci, a number considered typical for mammals (Lee *et al.* [2006](#)). Fruit flies have about 14 000 protein coding loci. Plants have diverse numbers of such loci, due in part to variable numbers of polyploid events in their evolution (Stokstad [2006](#)). *Arabidopsis* possesses about 27 000 loci from 11 000 gene families (Arabidopsis Genome Initiative [2000](#)). Estimates of the numbers of protein coding loci are not precise, as different computer algorithms for predicting coding sequences yield somewhat different answers.

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There are additional functional sequences that do not code for proteins

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Additional functional DNA sequences, including rRNA loci in large multigene families, tRNA genes (generally in small families), and small regulatory non-coding RNA (**ncRNA**) molecules are transcribed, but not translated (Moulton [2005](#)). Some of these (interfering RNA: **RNAi**) are involved in defences against viral infections and transposons (Dalmay [2006](#)). The number of ncRNAs in animals has yet to be documented, but about 75

000 expressed short RNAs have been identified in *Arabidopsis* (Lu *et al.* 2005). Other sequences are neither transcribed nor translated, including regulatory sequences, especially those in the 5' region before the transcription start sequence of protein coding loci.

These additional functional sequences increase the size of the region that can mutate to produce deleterious alleles (**mutational target**) to well beyond the size of the protein coding loci. They may also be involved in adaptive evolutionary changes. For example, short ncRNAs (19–24 nucleotides long) have been implicated in environmental adaptation in both plants and animals (Dalmay 2006). Micro RNAs (**miRNA**) are short non-coding RNAs, found in many plants and animals, that often act to post-transcriptionally inhibit gene expression. For example, a large difference in muscularity between sheep breeds is due to changes in the 3' untranslated region (3' UTR) of the myostatin locus which create a target site for a miRNA, resulting in translational inhibition (Cloup *et al.* 2006). Analysis of SNP databases for humans and mice demonstrated that mutations creating or destroying putative miRNA target sites are abundant and might be important effectors of phenotypic evolution (Cloup *et al.* 2006).

Further, many transcripts are not associated with any known functional element. About 20% of transcription in fruit flies is unassociated with protein coding loci and not explained by other known functional elements, while in humans 23% of transcripts are from intergenic regions (*Drosophila* 12 Genomes Consortium 2007; The Encode Project Consortium 2007).

## Copy number variation

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The probability of expressing a deleterious allele in the phenotype depends on copy number for the locus

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The probability of expressing a deleterious mutation in the phenotype depends upon whether a locus is present in one, two, three, four copies or more. A recessive mutation with a frequency of  $q$  in a random mating population will have a probability of being expressed in the phenotype of approximately  $q$ ,  $q^2$ ,  $q^3$  and  $q^4$  in individuals with one, two, three or four copies of the locus ([Chapter 4](#)). What proportion of loci shows copy number variation?

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There is widespread variation in gene copy number between and within species

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Losses and gains in gene number are evident within and across species. This applies to protein coding loci, other transcribed loci such as rRNA and non-transcribed sequences such as pseudogenes and microsatellites. Copy number variation has been found in a range of eukaryotes, including humans and fruit flies, and is likely to occur in all eukaryotes. In humans, 14.2% of coding loci vary in copy number (Nozawa *et al.* [2007](#)). Random pairs of individuals differ by an average of 61.5 copies of loci. In a sample of 270 individuals, 1447 copy number variable regions (1 kb or larger in size) were found and about half of the variants were present in more than one individual (Redon *et al.* [2006](#)). The variant regions contained hundreds of loci, functional units and segmental duplications. The copy number variation differed among human populations.

In humans, 541 deletion variants have been identified and  $\sim\frac{1}{2}$  were found in multiple unrelated individuals (McCarroll *et al.* [2006](#)). These vary in

length from 1 kb to 745 kb. Coding exons of 10 expressed loci were commonly deleted, including loci involved in steroid metabolism, olfaction and drug responses.

Copy number variation is particularly evident in multigene families, such as rRNA, globin and MHC loci. Multigene families change in copy number due to replication slippage and unequal crossing-over.

The fitness effects of copy number change vary. Some gene duplications are deleterious (*Bar* eye duplication in fruit flies), while others, such as duplication of the amylase locus in humans on high starch diets, are beneficial (Perry *et al.* 2007). Reductions in copy number of haemoglobin loci (thalassaemia) and the glucose-6-phosphate dehydrogenase locus in humans show heterozygote advantages in malarial areas (Chapter 9). Only a small minority of loci in fruit flies are highly deleterious or lethal when present in one or three doses (Lindsley *et al.* 1972).

## What proportion of genomes do transposable elements represent?

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Mobile DNA elements are the most abundant component of most eukaryote genomes and their movement can create mutations

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Transposable elements (mobile genetic elements) represent substantial proportions of eukaryote genomes (Table 10.2). The actual proportion varies among taxa, but in many it approaches 50% or more. Transposable elements are fragments of DNA that can insert into new chromosomal locations, often making duplicate copies of themselves in the process (Wessler 2006). These ‘DNA parasites’ are the cause of many mutations by inserting within or near

coding loci ([Chapter 7](#)). Further, they generate duplications and deletions by recombination between elements. Insertions of transposable elements are responsible for over 50% of spontaneous mutations in fruit flies, for about 10% in mice, but only ~0.1% in humans (Maksakova *et al.* [2006](#)). These differences may reflect propensities of the transposons to move in different species.

**Table 10.2** *Proportions of transposons in genomes of different species*

Species	Transposons contribution to genome
Humans	48%
Mice	40%
Frogs	77%
Fruit flies	15–22%
Plants	15% to over 90%
Bacteria	0.3%

Sources: Biémont & Vieira ([2006](#)); Sabot & Schulman ([2006](#)).

## Origins of genome complexity

Species differences in the number of duplicate genes, introns and mobile elements are related to effective population sizes, with greater numbers being found in species with lower population sizes

Genome sizes differ widely and are only weakly related to organism complexity. For example, the lungfish has 40 times as much DNA as mammals (Nei [1987](#)). Several adaptive hypotheses have been proposed to explain this paradox, but there is little support for them.

A novel extension of the near neutral theory of molecular evolution

(Chapter 9) has been its application to evolution of genome size and structure, especially locus duplications, introns and mobile elements. Lynch (2007) predicted that each of the above elements would be more likely to accumulate in species with smaller, rather than larger, effective population sizes. Some copy number changes will be mildly deleterious, but they more likely to be effectively neutral in species with smaller effective population sizes and to accumulate in them. As predicted, each of these variants is more common in species with smaller  $N_e$ . Whilst this theory remains controversial, it has made several predictions that have been supported in experimental tests.

## Insights into evolution from genomics

One of the most promising applications of genomics is determining the adaptive value of variation at loci across the genome (Chapters 6 and 9). Comparing genetic diversity among different regions within loci (coding versus non-coding; different positions within codons), across loci within species and among related taxa, provides insight into the selective nature of genetic diversity (neutral, advantageous or deleterious). The following sections consider how genomics can help identify loci subject to different kinds of selection.

### What proportion of loci is subject to directional selection?

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A minority of functional loci are probably subject to directional selection, but we are uncertain as to the exact proportion

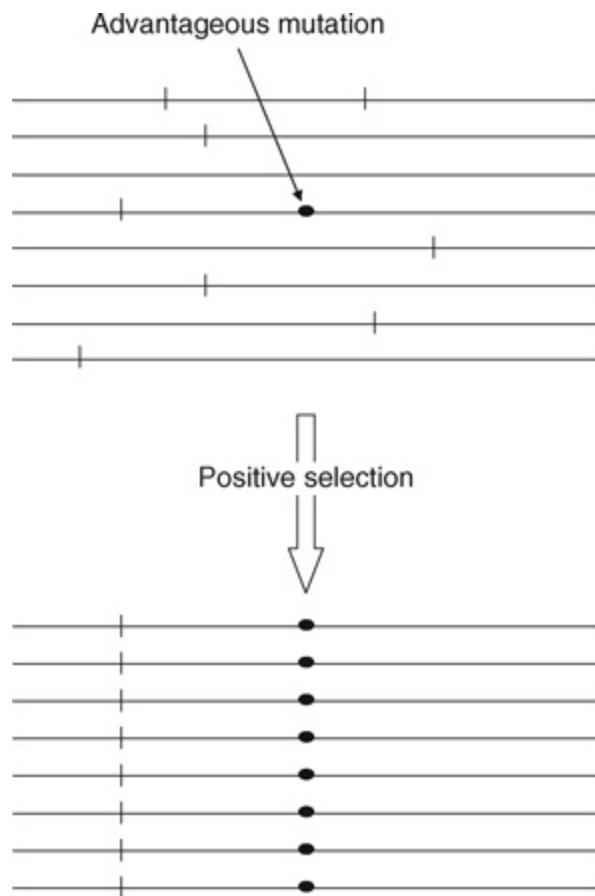
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At the genomic level, comparisons of behaviour among loci are widely

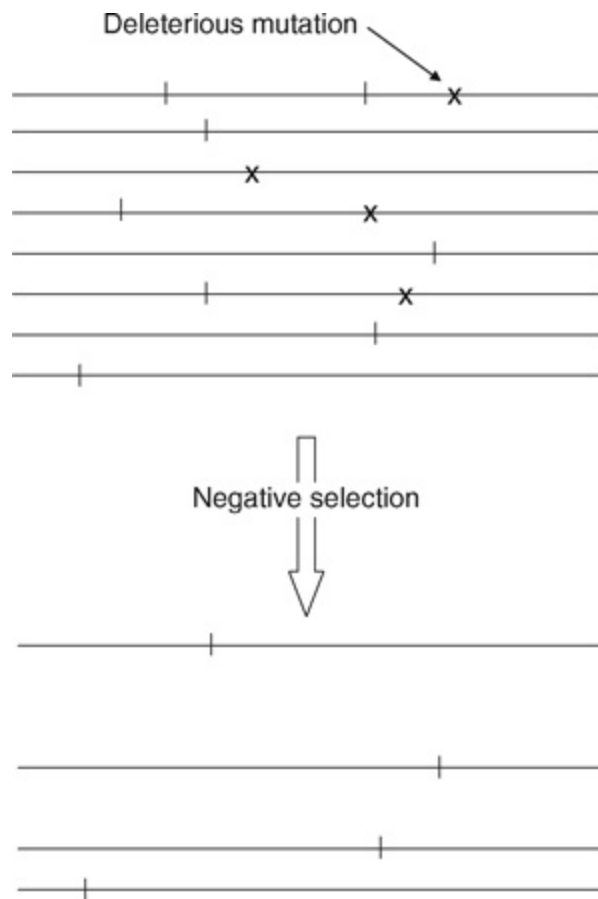


used to increase statistical power of analyses. Outlier loci, which behave in ways significantly different from the majority of loci, may be subject to selection. A range of statistical tests is available to detect such outliers (Luikart *et al.* 2003). There are five main ways for detecting adaptive selection from genomic analyses (Sabeti *et al.* 2006):

- High rates of non-synonymous (amino acid) substitutions in a locus compared to background rates, as found for loci in the human MHC. This is determined by DNA sequence comparisons with another species, such as chimpanzees in this case
- Reductions in genetic diversity in a region of the genome, compared to other regions within the same species. This indicates that an allele at a locus is being driven to fixation by selection and in the process reducing genetic diversity in surrounding regions (a **selective sweep** or **hitchhiking**: see Fig. 10.2). A selective sweep has occurred around the lactase locus, due to selection for alleles causing lactase persistence that confer the ability to metabolize milk in adult Europeans and dairying people from Africa (Tishkoff *et al.* 2007). These must be delineated from weak **background selection** due to removal of new deleterious mutations (Fig. 10.3)
- High frequency of non-ancestral, derived alleles. This has been found around the Duffy red cell antigen locus in Africans, presumably due to selection for malarial resistance. The ancestral allele has to be inferred from comparison of DNA sequences for the locus with those in closely related species.
- Large variation in allele frequencies among different populations of the same species, compared to variation at other loci, may signal a locus that has undergone positive selection in individual populations. The FY\*O allele at the Duffy locus in humans is near fixation in sub-Saharan Africans, due to selection for malarial resistance, but rare in other parts of the world.
- Linkage disequilibrium for a long region of DNA. This is also found around the lactase locus (Tishkoff *et al.* 2007).



**Fig. 10.2** Selective sweep due to a new adaptive mutation (after Nachman 2006). Each line represents a chromosome and short vertical lines represent neutral variants at nearby loci. *The rise to fixation of an initially rare allele reduces genetic diversity in both the selected locus and nearby ones. This also generates linkage disequilibrium.*



**Fig. 10.3** Background selection. This describes the effect on nearby neutral variation of selective removal of new deleterious mutations (after Nachman 2006).

Conditions in captive populations (including those of threatened species) are very conducive to selective sweeps, as they adapt to captivity. Selective sweeps throughout the genome have been found in 23 fruit fly populations brought into captivity and managed over 50 generations as recommended for endangered species in captivity (Montgomery, Woodworth, England, Briscoe & Frankham, unpublished data). Compared to neutral predictions, loss of microsatellite heterozygosity was 12% faster (Fig. 11.2), variation in microsatellite allele frequencies across replicates 25% greater, and temporal changes in allele frequencies 33% greater. Such impacts of hitchhiking are expected in long-term captive populations of threatened species.

The classic method for detecting selection at the molecular level for a

single locus is the McDonald–Kreitman test described in [Box 10.2](#). This method has also been the basis for several related tests.

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**Box 10.2 The McDonald–Kreitman test for selection (McDonald & Kreitman 1991; Hedrick 2005a; Eyre-Walker 2006)**

One of the most widely used tests for selection on molecular polymorphism was devised by McDonald & Kreitman (1991). The test compares the amount of variation within a species to the divergence (fixed differences) between species at synonymous sites and non-synonymous sites within the coding region of a locus. It assumes that synonymous substitutions are neutral.

If the observed non-synonymous variation is neutral, then the rate of base substitution (divergence) between species and the polymorphism within species are both a function of the mutation rate. Consequently, we expect the ratio of divergences between species for non-synonymous versus synonymous sites ( $D_n/D_s$ ) to equal the ratio of the polymorphism within the species for non-synonymous to synonymous sites ( $P_n/P_s$ ). However, if directional selection is occurring on non-synonymous sites, these contribute substantially to divergence between species, but little to polymorphism and so result in  $D_n/D_s > P_n/P_s$ . For example, for the fruit fly data below the ratio of divergences  $3648/7365 = 0.50$  is greater than the ratio of polymorphisms  $439/1741 = 0.25$ . Thus, the non-synonymous divergence is partially due to adaptive fixations. If the difference is in the opposite direction, as for primate mtDNA coding loci, then it is presumed that most non-synonymous polymorphisms within species are slightly deleterious and are eliminated before they become fixed in different species.

Molecular polymorphism data from 115 loci in two species of fruit flies

	Divergence	Polymorphism
Non-synonymous	3648	439
Synonymous	7365	1741

This information can be used to estimate the proportion of non-synonymous substitutions that are adaptive ( $\alpha$ ), assuming all synonymous ones are neutral, as:

$$\alpha = 1 - \frac{D_s P_n}{D_n P_s}$$

and by substituting from the table above,

$$\alpha = 1 - \frac{7365 \times 439}{3648 \times 1741} = 0.49$$

Thus, about half of the amino acid substitutions between fruit fly species have been adaptive substitutions.

Seven human studies using the above genomic methods have not yielded consistent estimates of loci under selection, and fewer than half of the loci previously identified as targets of positive selection ranked among the top candidates in these assays (Sabeti *et al.* 2006).

In another study only 1.6% of the SNPs examined showed a signature of selection for beneficial alleles (positive selection) using a linkage disequilibrium decay test (Wang *et al.* 2006). The ~ 1800 loci identified were predominantly involved in host–pathogen interactions, reproduction, DNA metabolism/cell cycle, protein metabolism and neuronal function. Adaptive changes comprise only a small minority of the DNA sequence differences between humans and chimpanzees (Nielsen *et al.* 2005). Loci showing evidence of selection were predominately involved in sensory perception,

immune defence, tumour suppression, apoptosis and spermatogenesis. Thus, only a small proportion of SNP variation is being subject to natural selection in hominids.

Some authors have suggested that modern humans are subject to little selection, as mortality rates are low and family size variation is constrained by contraception, a situation reminiscent of genetically managed captive populations of threatened species. However, a test on 1.2 million SNPs in Chinese, European–American and African–American populations revealed signals of recent positive selection, with about 101 showing very strong evidence for selective sweeps (Williamson *et al.* 2007). More signals were population specific than shared across groups. A few of the signals correspond to loci known to have been recently selected, including the lactase locus in Europeans, and the alcohol dehydrogenase locus in Chinese. There were also selective sweeps involving several centromeres.

Searches for regions subject to balancing selection in humans have been carried out by identifying regions with higher genetic diversity than the rest of the genome, and by exploring for ancient polymorphisms shared between humans and chimpanzees (Asthana *et al.* 2005; Bubb *et al.* 2006). Both studies indicated very low proportions of balanced polymorphisms in the human genome.

## **What proportion of amino acid substitutions is deleterious?**

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A majority of amino acid substitutions are deleterious and the proportion is higher in species with smaller effective population sizes

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The ratio of base substitutions that result in amino acid substitutions ( $K_A$ )

to synonymous substitutions ( $K_S$ ) provides a measure of the proportion of amino acid substitutions that is deleterious. This is similar to  $D_n/D_s$  in the McDonald–Kreitman test (Box 10.2), but applied to amino acids, rather than nucleotides. In humans  $K_A/K_S$  is 0.23, implying that 77% of amino acid alterations in humans are sufficiently deleterious to be eliminated by natural selection (The Chimpanzee Sequencing and Analysis Consortium 2005). The  $K_A/K_S$  ratio in murids is only 0.13, indicating stronger functional constraints in murids (87%) than in hominids. Presumably negative selection is less effective in hominids due to their lower long-term effective population sizes. Estimates of the distribution of fitness effects for amino acid substituting mutants in humans indicate an average reduction in fitness of a few per cent, with >50% having mild effects and <15% having strongly deleterious effects (Eyre-Walker *et al.* 2006).

Only a small proportion of amino acid substitutions in humans is due to positive adaptive selection. An analysis of 8079 loci showed that 0.4% had  $D_n/D_s$  ratios significantly >1, as expected with positive selection (Nielsen *et al.* 2005) (Box 10.2).

## Does selection act on non-coding DNA?

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At least some non-coding DNA is subject to selection

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It is possible to test for selection on non-coding DNA sequences by comparing their polymorphisms and rates of evolution with those for synonymous sites. Such tests on non-coding DNA in fruit flies reveal evidence of selection on UTR, introns and intergenic DNA (Andolfatto 2005). All of these evolve more slowly and are less polymorphic than

synonymous sites. About 20% of the nucleotide divergence in introns and intergenic DNA is probably driven to fixation by positive selection and about 60% for UTR. Considering both positive and negative selection (constraint), about 83% of the nucleotides in UTR are functionally relevant. The values for introns and intergenic regions are about 46% and 60%, respectively. In humans, 98.5% of regions that are highly conserved across mammals lie outside protein coding sequences (Pollard *et al.* 2006). This is in line with earlier evidence that the major determinants of differences between humans and chimpanzees are in regulatory regions of the genome (Carroll 2005). Another study indicates that about two-thirds of all sequences subject to selection against deleterious alleles (**purifying selection**) in the human genome are non-coding. In rats and mice, there are more than three times as many selectively constrained, non-repetitive sites within non-coding DNA than in coding DNA and this is similar in a variety of vertebrates (Gaffney & Keightley 2006). Substantial fractions of non-coding regions of invertebrate genomes are also selectively constrained, but the fraction is lower than for coding regions (Siepel *et al.* 2005). Both regulatory sequences and coding regions contribute to evolutionary changes, but those in regulatory sequences may be the more important.

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Cis-regulatory sequences are constrained by stabilizing selection

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Regions at the 5' end (cis) of protein coding loci contain regulatory sequences including binding sites for transcription factors. These cis-regulatory sequences are constrained by natural selection. For example, sea urchins cis-regulatory regions contain almost no small insertions and deletions (indels), a signal of functional importance (Cameron *et al.* 2005). Similar selection is expected for regulatory sequences in all species.



## Are synonymous variants neutral or subject to selection?

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Synonymous variants show evidence of being subject to very weak selection across a broad range of taxa

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It is frequently assumed that synonymous variants are neutral (see above and [Chapter 9](#)). However detailed analyses of genomes have revealed evidence for weak selection operating on synonymous variants in yeast, fruit flies, *Arabidopsis* plants, nematodes and humans (Comeron [2006](#)). This occurs because preferred codons have greater translational efficiency than non-preferred synonymous codons (Carlini [2004](#)). The selection on synonymous variants is typically so weak (average  $s \sim 10^{-6}$ ) that it will be swamped by drift in threatened species (Carlini [2004](#)).

## Size of the mutational target

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Genomic deleterious mutation rates depend upon the total size of functional elements in the genome and upon their rate of mutation

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Inbreeding depression is a major concern in conservation biology. It is primarily due to homozygosity for deleterious alleles in mutation–selection balance ([Chapter 13](#)). The total magnitude of inbreeding depression depends predominantly on the number of loci that can mutate to produce deleterious

mutations and their mutation rates. The size of the deleterious mutation target is determined by the number and size of coding loci plus the functional sites in non-coding regions. The **mutational target size** for deleterious mutations is clearly less than the total size of the genome and larger than the total of all protein coding loci. From the conserved sequences in the mouse genome, this may represent only ~5% of the genome (Anonymous [2007](#)).

The rate of mutation depends upon the rate of spontaneous DNA replication errors and upon the rate of transposon-induced mutations. The array and activity of transposons indicates their potential impact as mutagenic agents.

Estimates of whole genome mutation rates have been made in fruit flies (Haag-Luitard *et al.* [2007](#)), yielding a mutation rate/base of  $8.4 \times 10^{-9}$  per generation. This indicates a total genome deleterious mutation rate of 1.2 per diploid genome per generation, a relatively high rate with implications regarding mutation–selection balance and inbreeding depression.

## Insights from gene expression studies

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There is extensive heritable variation in gene expression levels among individuals within species

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Variation in transcription levels among individuals is widespread, having been documented for humans, mice, fish and invertebrates (Ranz & Machado [2006](#)). Expression variation among individuals has a large heritable component (Whitehead & Crawford [2006a](#)). Replicate inbred populations (homozygous but genetically different) within species differ in expression at

many loci in mice, fruit flies and maize (Whitehead & Crawford [2006a](#)). In addition to differences in cis-acting regulatory regions near protein coding loci, polymorphisms in many other loci affect expression of particular loci.

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Adaptive differences in gene expression have been identified between populations within species

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Loci with genetic differences in transcription levels among populations may be involved in adaptive differences (Ranz & Machado [2006](#)). For example, gene expression at 329 loci involved in central metabolic pathways in the killifish *Fundus heteroclinus* were tested in a common garden experiment, involving five populations from a temperature gradient between Maine and Georgia in the Eastern USA (Whitehead & Crawford [2006b](#)). This identified 44 loci (13%) that appeared to be subject to natural selection (4% directional selection, 7% stabilizing selection and 2% balancing selection). Outlier loci with significantly greater differences than average differences in gene expression among populations are likely to be subject to directional selection in diverse directions among populations. Conversely, expressions that differ less than average among populations provide a signal for loci experiencing balancing or stabilizing selection. Microarray studies are also being undertaken to identify loci involved in hybrid sterility and speciation (Ranz & Machado [2006](#)).

## **Genetic basis of hybrid vigour**

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Hybrid vigour is due predominantly to dominance of favourable alleles, with a minor component due to heterozygote advantage

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A major tool for rescuing small inbred populations of threatened species is to outcross them to another population ([Chapter 13](#)). This is of major importance in managing fragmented populations ([Chapter 17](#)). When inbred populations are crossed, the  $F_1$  progeny typically show increased reproductive fitness termed **hybrid vigour** or **heterosis**. The genetic basis of this has been a matter of controversy since early last century, with dominance of favourable alleles and heterozygote advantage being the main competing hypotheses. Resolution of this issue will assist us to understand the genetic architecture of fitness and to predict effectiveness of natural selection in purging deleterious alleles ([Chapter 13](#)).

Expression microarrays containing about 14 000 cDNAs were used to compare mRNA levels in two inbred maize parents and their  $F_1$ . Of the cases of non-additive gene action that contribute to heterosis, 70% showed dominance for gene expression in the higher yielding parent, 13% showed overdominance and the remainder showed dominance for the lower yielding parent, partial dominance or underdominance (Swanson-Wagner *et al.* [2006](#)). Dominance was the main form of gene action contributing to heterosis, but overdominance was also a significant contributor. The former component, but not the latter, is subject to removal (purging) by natural selection ([Chapter 13](#)).

Below, we apply genomic information in discussing the prospects for individual-locus genetic management in threatened species.

## Prospects for individual-locus genetic management

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Polymorphisms with large effects on fitness are known at various loci. For some, individual genetic management may be justified, whilst managing the remaining variation based upon neutral predictions

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As population genomics allows us to detect loci that are subject to selection and to determine the forms of selection operating, we could potentially augment genetic management by concentrating partly on these loci, whilst managing the remainder of the genome to maximize retention of genetic diversity. Tasmanian devils in Australia may require management of the MHC separately from the remainder of the genome, as they are at serious risk of extinction in the wild due to a tumour that is spread by biting ([Box 10.3](#)). Fortunately, there appears to be genetic resistance in some animals due to different MHC haplotypes. The resistant haplotypes may need to be managed in the captive population and could be important in any subsequent reintroductions.

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**Box 10.3 Separate management of the MHC in Tasmanian devils (Jones et al. [2004](#); McCallum & Jones [2006](#); Siddle et al. [2007](#))**



## Tasmanian devil

Recently, a case indicating potential separate genetic management of the MHC has arisen in the Tasmanian devil. The devil, a dog-sized marsupial carnivore, is suffering a wave of extinction across the island of Tasmania in Australia due to a clonal tumour transmitted from affected to unaffected animals by biting ([Chapter 2](#)). Over much of Tasmania, devils are so similar at the MHC that they do not reject the devil-derived tumour tissue. However, preliminary evidence indicates that there are some animals in the northwest of Tasmania that have different MHC alleles and are more resistant to the tumour.

Given the high risk of extinction for the species in the wild, a captive population has been founded. Normally such a population would be managed at the whole genome level to maintain maximum genetic diversity using methods described in [Chapter 19](#). However, in this case it could be desirable to increase the frequency of the resistant MHC haplotype in the population, in the hope that resistant devils can be reintroduced to the wild. Simultaneously, it will be desirable to maximize retention of genetic diversity in the remainder of the genome. The genetic management plan has yet to be devised, but the discussion below indicates some of the issues and options that need to be considered and possible solutions.

The management of the MHC will depend in part on whether tumour resistance is dominant or recessive (dominance is likely). It would be unwise to make the resistant MHC haplotype homozygous, as resistance to other diseases might be lower in this genotype. All major MHC haplotypes will need to be conserved to cope with future disease challenges. At what frequencies should the MHC haplotypes be maintained? One option is to increase the tumour resistant haplotype to a higher frequency (say 0.5). If as expected resistance is dominant, this would yield 75% resistant individuals under random mating, possibly sufficient to allow survival of reintroduced populations even in the presence of the disease. The other haplotypes would best be managed at equal frequencies in the absence of other relevant information. An option for the management of genetic diversity in the remainder of the genome would be to manage for maximum microsatellite diversity, in a manner related to that being done in whooping cranes ([Jones \*et al.\* 2002](#)).

Fortunately, crossing resistant devils from the northwest with devils from elsewhere will both increase the frequency of the resistant haplotype and increase microsatellite genetic diversity in the captive population. If the resistant haplotype is at a high frequency in the northwest, a conceptually simple approach would be to cross all devils sourced from elsewhere to resistant homozygotes (using as large a sample as possible) to generate a resistant haplotype frequency of 0.5, whilst also maximizing microsatellite variability. Subsequent management could then focus on within-family selection to maintain the resistant haplotype at a frequency of 0.5, while maximizing retention of whole genome (microsatellite) diversity via between-family management. When animals are reintroduced, the possibility exists of using only genetically resistant heterozygous animals, if the disease persists in small pockets in the wild.

Detailed new methods will need to be devised to combine all the necessary objectives of such genetic management programs. Analytical studies and computer simulations will be critical tools in optimizing the genetic management of the Tasmanian devil captive population. It will also be critical to determine the frequency of the resistant MHC haplotype in the devil population from the northwest and to determine whether resistance to the tumour is dominant or recessive. Recently, the tumour appears to have evolved an elevated mutation rate. Consequently, the tumour may evolve means to overwhelm host resistance, calling into doubt any efforts to manage the MHC.

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However, there are important reservations about individual-locus genetic management. The first group of issues arise primarily due to sampling variation and statistical power. Some of the apparently selected loci will be neutral, or have the form of selection incorrectly diagnosed, or will be linked to selected loci, rather than selected themselves. The second problem is that some loci will have different patterns of selection in diverse environments, including future environments. For example, alleles favoured in captivity are often deleterious in the wild (Frankham [2008](#)).

In rare cases, apparently beneficial alleles would actually be deleterious, so that individual management on the locus will be deleterious. Confirming selection on candidate loci is necessary, but is a difficult, expensive and time-consuming process. The evidence for selection on a candidate locus is strengthened if there are positive answers to the following questions:

- Does the locus function in the correct tissue at the correct time? For example, does an allele presumed to confer the ability for adults to digest lactose function in the digestive system of adults?
- Is the favoured allele correctly transcribed and translated?
- Do gene products of apparently favoured alleles differ in the predicted direction? For example, does an amylase allele involved in adaptation to a starchy diet increase amylase enzyme activity, rather than decrease it?
- Does the candidate locus have the hypothesized effect in another species? For example, MHC and self-incompatibility loci show molecular signals of balancing selection in many species ([Chapter 9](#)).
- Does a transgene containing a favoured candidate allele have beneficial phenotypic effects when inserted into the species, compared to a transgene containing the presumed deleterious allele?

In model species such as fruit flies and mice, evaluation of the effects of introducing a transgene containing the candidate loci into progeny is typically considered necessary to confirm causality of a particular effect (Daborn *et al.* [2002](#)). However, this will rarely, if ever, be feasible in threatened species. In these species, putative selective loci are likely to be ‘confirmed’ by consistency of a range of lines of evidence, including the first four questions above. The above discussion leads us to recommend a cautious approach to single-locus genetic management.

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Some of the alleles responsible for inbreeding depression will be



identifiable using population genomics and it may be desirable to select directly against some of them

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In theory we should be able to define the deleterious alleles responsible for inbreeding depression. This holds out the prospect of deliberately selecting against them, and thus reducing the susceptibility of populations to inbreeding. In humans, bioinformatics has been used to predict the consequences of non-synonymous SNPs on proteins (Kohn *et al.* 2006). At the extreme, variants that result in stop codons within protein coding regions will typically be deleterious alleles. Further, variants that substitute amino acids in functional sites within proteins or transcription factor binding sites within promoters are likely to have functional significance.

In this vein, Kristensen *et al.* (2005) compared transcription levels in outbred and inbred populations of fruit flies using microarrays and identified 67 loci with consistent changes in expression. Many of these loci were involved in metabolism and stress response and overlapped with loci affected by ageing and oxidative stress.

Currently it is not practical to detect and remove these deleterious alleles across the genome.

## **Implementing individual-locus genetic management**

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Individual-locus genetic management could be applied at the within-family level, whilst still managing for maximum genetic diversity overall based on between-family choice of individuals to breed

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Before moving to implement individual-locus genetic management it is crucial to have established that the alleles to be managed individually have consistently beneficial (or deleterious) effects in the current and future wild environments. Since detailed single-locus management will typically only be done in captivity, this requires that the allele has the same direction of beneficial (or deleterious) effects in both captive and wild environments.

What loci might justify individual management? First, MHC loci in vertebrates, self-incompatibility loci in plants and sex loci in Hymenoptera are obvious candidates. Selection for diversity at MHC loci has already been suggested (Hughes 1991), but this cannot be recommended as a general approach until there is much greater understanding of the forces of selection operating on the MHC. However, the case of the Tasmanian devil may require separate management of the MHC (Box 10.3). Second are particular deleterious alleles that have reached high frequencies due to drift in small threatened populations, such as the chondrodystrophy allele in California condors (Chapters 4 and 19). It will be feasible to eliminate such recessive alleles once heterozygotes are identifiable. Such cases are likely to be among the first direct use of genomics in conservation genetics.

Implementing individual-locus genetic management may detract from overall management to retain genetic diversity, unless considerable care is taken. One approach that minimizes such problems is to apply individual-locus management within families, whilst still managing for maximum overall genetic diversity by between-family choice of breeders. This could be implemented for chondrodystrophy in the California condors. Breeders would be chosen by minimizing mean kinship (Chapter 19). Within the chosen families, only individuals that were homozygous normal at the chondrodystrophy locus would contribute to the next generation.

Computer simulations of the individual-locus management regimes in concert with minimizing mean kinship are required before such approaches are applied to threatened species. These will need to define the risks of

misdiagnosing selection on loci, for different numbers of individuals and different sequencing redundancies. Following this, the practice needs should be checked on model organisms, such as fruit flies, mice or *Arabidopsis*. In a related vein, marker-assisted selection is being applied in domestic animals and plants and this should provide guidance with respect to the possible benefits of individual-locus genetic management for threatened species (Tuberosa & Salvi [2006](#)).

The prospects of individual-locus management will have to be weighed against the cost of genotyping. Cost and accuracy concerns will mean that individual-locus management is only likely to be applied to a relatively small number of loci that exhibit the most convincing cases for being selected. We expect that individual-locus management will only be an adjunct to broadly based genome management in the foreseeable future.

## Summary

1. Many new insights into genome evolution have resulted from sequencing of whole genomes and characterizing DNA sequence variation within species.
2. Sequence information can be used in microarray technology that allows simultaneous measurement of gene expression across the genome.
3. Whilst sequencing is currently too expensive to be feasible for most threatened species, rapidly reducing costs should allow more high-profile threatened species to be sequenced.
4. Population genomics provides detailed information on genetic diversity for coding loci, for non-coding sequences and for copy number variation.
5. Population genomics has provided important insights into genome evolution, including proportion of loci subject to selection, proportion of amino acid substitutions that are deleterious, extent of selection on non-coding DNA and the size of the target for deleterious mutation.
6. Genomic studies allow us to detect multilocus impacts, including

selective sweeps, background selection and associative overdominance.

7. Among the first uses of genomics in conservation genetics is likely to be to devise carrier detection tests for single-locus genetic diseases, such as chondrodystrophy in California condors, by identifying the locus in related sequenced species.
8. Population genomics in threatened species may allow genome-wide management of heterozygosity to be augmented with some individual-locus management. However, inaccuracies in delineating selected loci and alleles requires that considerable caution be used in applying such techniques. Individual-locus management is only likely to be justified for a small number of loci and alleles where evidence for similar selection in captivity and the wild is very clear.

### **Further reading**

Kohn *et al.* (2006) Brief review on genomics and conservation genetics.

Lee & Mitchell-Olds (2006) Brief review on ecological and evolutionary genomics of populations in nature.

Luikart *et al.* (2003) Review on population genomics, with emphasis on statistical tests for detecting outlier loci.

Ranz & Machado (2006) Review of methods and evolutionary studies on gene expression using microarrays.

Ryder (2005) Reviews on potential impacts of whole genome sequencing and expression microarray studies in conservation of threatened species.

van Straalen & Roelofs (2006) *Introduction to Ecological Genomics*. Textbook on population genomics.

### **Software**

BLAST: Provides sequence similarity searches of sequence databases. Included in Genbank (see below).

DNA<sub>SP</sub>: Software for statistical testing for selection. [www.uc.es/dnasp/](http://www.uc.es/dnasp/)

GARFIELD: Genome annotation resources for the domestic cat genome sequence, a sequence that genome enables threatened cats (Pontius & O'Brien 2007). <http://lga.abcc.ncifcrf.gov/>

GENBANK: Central repository for DNA sequence data (Benson *et al.* 2007). [www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)

PAML 4: Programs for analyses of molecular data to estimates phylogenies, calculate  $D_n$  and  $D_s$ , detect of loci subject to selection, estimate species divergence times and reconstruct ancestral sequences (Yang 2007). <http://abacus.gene.ucl.ac.uk/software/paml.html>

## Problems

- 10.1 What is a genome?
- 10.2 What is a genome-enabled species?
- 10.3 What is a microarray?
- 10.4 What is miRNA?
- 10.5 What is the transcriptome?
- 10.6 In what ways can nearby linked loci influence the evolution of a particular locus?
- 10.7 Use the McDonald–Kreitman test to interpret the MHC (HLA-B) differences within and between humans and chimpanzees (Hedrick 2005a p.440).

	Divergence (fixed)	Polymorphic
Non-synonymous	0	76
Synonymous	0	49

## **Section II Effects of population size reduction**

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Threatened species have small, or declining populations. Once small, they lose genetic diversity, become inbred (with consequent reduction in reproductive fitness) and accumulate deleterious mutations. Consequently, Section II considers these factors in detail, as they contribute to extinction risk, and provide the essential background material for the genetic management of threatened species in [Section III](#).

### **Factors reducing population size**

Humans are reducing the size and distribution of wild populations through clearing and fragmentation of habitat, over-exploitation, pollution and the impact of introduced species. Of these, habitat loss is currently having the greatest impact, but threats from global climate change loom ever larger.

### **Loss of genetic diversity**

Loss of genetic diversity in small populations reduces the ability to evolve in response to ever-present environmental change. There are four threats to genetic diversity:

- extinction of populations or species
- extinction of alleles due to sampling in small populations
- inbreeding reducing heterozygosity by redistributing genetic diversity among homozygous individuals and populations

- selection favouring one allele at the expense of others, leading to fixation.

Overwhelmingly the major threat to genetic diversity is extinction of alleles in finite populations by genetic drift. All of the adverse genetic effects of population size reduction depend on the effective population size, rather than the census size. The effective population size is reduced by fluctuations in population sizes, high variation in family sizes, and by unequal sex-ratios. **Chapter 11** deals with the effects of small population size on genetic diversity and the factors that influence effective population size. It also deals with coalescence and gene tree analyses that introduce a time (generations) dimension and often allow more information to be gleaned from analyses.

## **Inbreeding**

Inbreeding is an inevitable consequence of small population size. Eventually every individual becomes related, so that matings amongst relatives cannot be avoided. **Chapter 12** describes how inbreeding is measured, and its rate of increase in finite populations. Inbreeding exposes deleterious mutations and reduces reproductive fitness and so increases extinction risk. **Chapter 13** documents the widespread occurrence of inbreeding depression, discusses its genetic basis and describes the use of outcrossing to reverse it.

## **Population fragmentation**

Habitat fragmentation reduces population sizes and increases isolation of population fragments. The impacts of population fragmentation depend critically on population structure and gene flow. Completely isolated population fragments suffer elevated rates of inbreeding and loss of genetic diversity, and consequently have elevated extinction risks compared to single populations of the same total size. **Chapter 14** deals with the genetic consequences of population fragmentation, with the means for measuring population differentiation and inferring gene flow, and with landscape

genetics.

## **Genetically viable populations**

The section concludes with **Chapter 15**, 'Genetically viable populations'. We ask: how large a population is required to avoid inbreeding depression? To avoid loss of evolutionary potential? To avoid accumulating deleterious mutations? These sizes are compared with actual population sizes for endangered species and size targets for delisting species. Current goals for genetic management of captive populations represent a compromise that recognizes that there will be modest genetic deterioration over time.



## Chapter 11 Loss of genetic diversity in small populations

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Population size reductions are the main reason for loss of genetic diversity. Rates of loss in closed populations depend on the effective population size ( $N_e$ ) and on the number of generations.  $N_e$  is usually much less than the number of adults in a population. Gene trees and coalescence analyses allow more powerful analyses of genetic diversity as they encompass a time dimension

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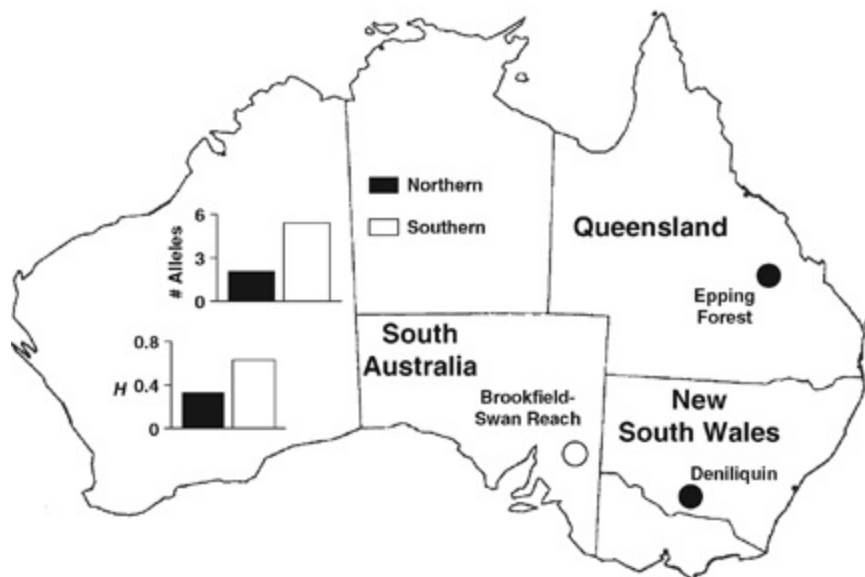
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### Terms

Coalescence, effective population size, gene trees, harmonic mean, idealized population, polyandry, polygyny

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Low genetic diversity in the critically endangered northern hairy-nosed wombat, compared to its nearest relative, the southern hairy-nosed wombat (after Smith & Wayne [1996](#))

## Changes in genetic diversity over time

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The current genetic diversity in a population derives from cumulative effects over many previous generations of mutation, natural selection and, especially of population size

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In [Section I](#), we explored the evolutionary forces which influence genetic diversity and contrasted their importance in small versus large populations. Our major conclusions are:

- genetic diversity provides the raw material for evolutionary adaptive change
- loss of genetic diversity is unavoidable in small closed populations
- mutation is the ultimate source of all genetic variation
- mutation and migration are the only mechanisms for restoring lost diversity; as mutation rates are very low, this factor is inconsequential for genetically depauperate endangered species
- single locus, quantitative and total genomic genetic diversity can be estimated by a variety of laboratory techniques
- some adaptive genetic variation is maintained within populations by balancing selection
- the influence of natural selection depends upon population size
- the fate of alleles in most small populations of endangered species is predominated by random factors
- inbreeding, with consequent loss of fitness, becomes inevitable in small populations
- effective population size ( $N_e$ ), as opposed to the observed census size, determines loss of genetic diversity and inbreeding.

For simplicity, we have primarily considered single-generation changes. However, the current genetic status of a population is a consequence of

cumulative effects over many previous generations, and our predictions of future changes must extend over many generations.

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Loss of genetic diversity in small populations is related to inbreeding, so a positive correlation is expected between population mean heterozygosity and population mean reproductive fitness

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At first it may seem that loss of genetic diversity is only of concern for evolutionary adaptation in the long term. However, there are often corresponding immediate short-term losses of fitness.

Concerns about loss of genetic diversity in conservation biology are actually concerns about both loss of evolutionary potential and of short-term loss of fitness due to inbreeding depression (see below and [Chapter 12](#)). Two meta-analyses have confirmed that the predicted relationship between population average genetic diversity and population fitness exist; the average correlation between population fitness and population average heterozygosity being 0.3–0.4 (Reed & Frankham [2003](#); Leimu *et al.* [2006](#)). Disease resistance/tolerance has also been shown to be related to genetic diversity in a range of animal and plant species (see [Chapter 2](#)).

## **Relationship between loss of genetic diversity and reduced fitness**

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Loss of alleles at self-incompatibility loci in small plant populations reduces population reproductive fitness

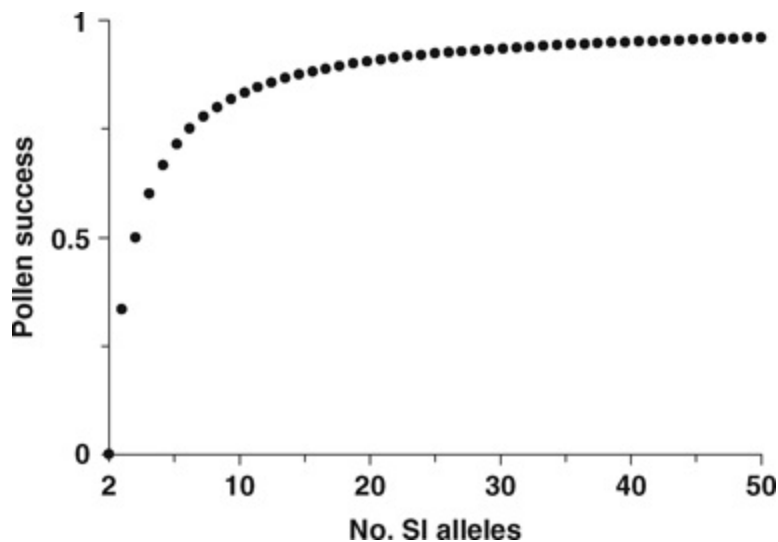
Particular relationships between loss of genetic diversity and fitness that are not due to inbreeding occur in self-incompatible species and in haplo-diploids. Losses of SI alleles in small self-incompatible plant populations reduce population fitness (Demauro 1993; Young *et al.* 2000; Castric & Vekemans 2004) (Box 11.1). All threatened self-incompatible plant species are susceptible to similar problems. Loss of genetic diversity at the sex locus reduces population fitness in haplo-diploid species, in a related manner (Zayed & Packer 2005; Box 17.5).

**Box 11.1 Relationship between loss of SI allele diversity and reproductive fitness in the endangered self-incompatible grassland daisy (Young *et al.* 2000; A. G. Young pers. comm.; Pickup & Young 2007)**

Loss of SI alleles in self-incompatible plants is expected to reduce both the proportion of pollen that can fertilize (figure below) and the proportion of ovules fertilized.



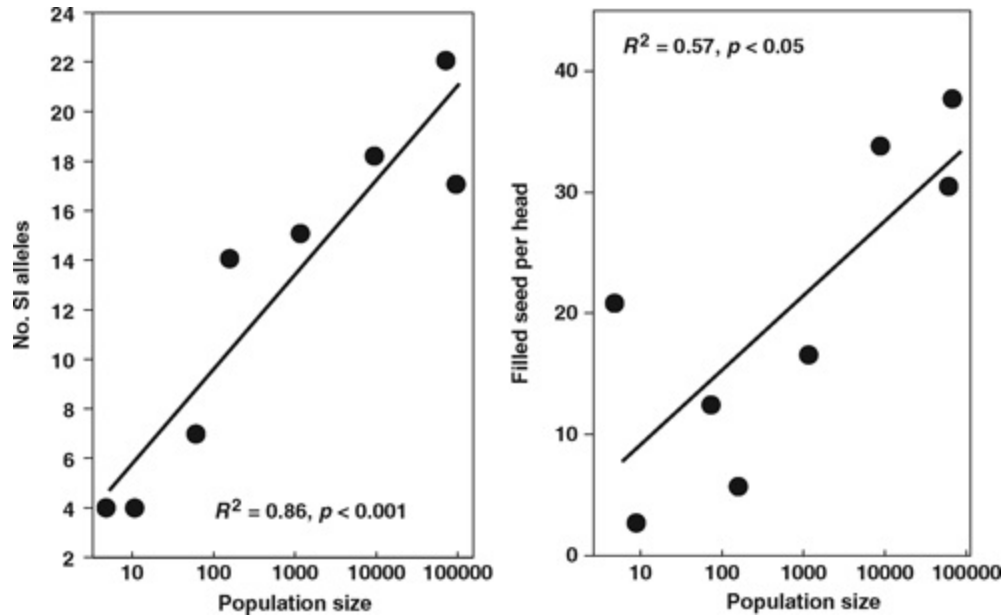
Endangered grassland daisy



Predicted relationship between maximum proportion of pollen that can fertilize and number of SI alleles in plants with gametophytic self-incompatibility.

Direct evidence of loss of SI alleles and reduced reproductive fitness has been found in small populations of the endangered grassland daisy in eastern Australia. SI allelic diversity (and allozyme diversity) declined with population size in five population fragments of the daisy with sizes from 5 to 70 000 plants. Number of seeds per plant was related to  $\log N$ . This relationship between seeds/plant and population size was not due to a shortage of pollinators in small populations as there were about 50 pollen grains per stigma and only a single ovule to fertilize. Use of pollen from other populations increased seed set in small populations, confirming that the small number of SI alleles in small populations

caused the reduced seed set.



The relationship between individual heterozygosity and individual reproductive fitness varies from positive to negative, unless inbreeding is present

The observed relationships between heterozygosity of individuals and their reproductive fitness within random mating populations are inconsistent, but positive relationships are most common (Deng & Fu 1998; Acevedo-Whitehouse *et al.* 2006). For example, a study of Glanville fritillary butterfly populations in Finland showed that allelic variation at the PGI locus affected population growth and that the direction and magnitude of effects changed with the area and spatial connectivity of habitat patches (Hanski & Saccheri

2006). By contrast, genotypic differences at six other loci did not correlate with population growth.

Whilst it is often assumed that overdominance is required for a positive relationship between individual heterozygosity and individual fitness, this is not necessary. A positive relationship occurs when the heterozygote fitness is greater than the weighted mean fitness of the two homozygotes at a locus (Deng & Fu 1998).

Below, we first consider the impact on neutral genetic diversity of small effective population size, sustained over many generations. Second, we consider the impacts of fluctuations in population size over generations, variable family sizes and unequal sex-ratio on effective populations sizes. Third, we review methods for estimating effective population size. Fourth, we introduce gene trees and coalescence analyses that add a time dimension to population genetic analyses. Details of the impacts of population size restriction on inbreeding are deferred until [Chapters 12](#) and [13](#).

## Effects of sustained population size restrictions on genetic diversity

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Genetic diversity is lost primarily from sustained restrictions in effective population size

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In [Chapter 8](#) we saw that loss of genetic diversity resulted from drastic reductions in population size (bottlenecks) over one or a few generations. However, serious loss of variation can accumulate over generations even with modest population restrictions. Population sizes are declining for many

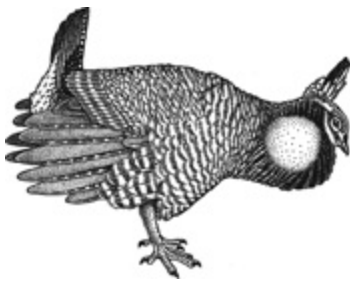


species across the globe, with an average reduction for vertebrates of 40% between 1970 and 2000 (Millenium Ecosystem Assessment [2005a](#)). A population of effective size 100 (a size typical for Vulnerable species – see below) loses 25% of its heterozygosity over 57 generations, the same loss as a single-generation bottleneck of one pair ([Fig. 8.5](#)). For example, the Illinois population of the greater prairie chicken dwindled from several million to fewer than 50 individuals over a 130-year period, with concomitant reduction in genetic diversity ([Box 11.2](#)).

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**Box 11.2 Loss of genetic diversity due to sustained population size reduction in the greater prairie chicken (Bouzat et al. [1998](#))**

Populations of the greater prairie chicken in Illinois were estimated to be in the millions in the 1860s, but subsequently declined to less than 50 in 1993. In contrast, populations in Kansas, Minnesota and Nebraska remained comparatively large (4000 or more).



Greater prairie chicken

The current Illinois population has fewer microsatellite alleles per locus than found in populations from Kansas, Minnesota and Nebraska. Further, it has lost alleles since 1960 (based on genotyping of museum specimens); all the alleles shown in bold below for the pre-1960 samples are now absent from the Illinois population.

Population	Locus						Allelic diversity
	1	2	3	4	5	6	
Illinois							
now	ABC-*	ABCD-	A--D-F-H	ABC---	-B--E--	--C-EFGHI---	3.67
pre-1960	AB--	-BCDE	A--D-F--	ABC-E-	-BCDEFG	-BCDEFG-I-L	5.12
Kansas	ABCD	-BCDEF	A-CDEFGH	ABCDE-	ABCDE--	ABCDEFGHI---	5.83
Minnesota	ABCD	ABCD--	ABCDEFGH	ABC-E-	-BCDE--	-BCDEFGHI---	5.33
Nebraska	ABCD	ABCDE-	ABCDEFGH	-BC-EF	-BCDE--	-BCDEFGHIJK-	5.83

\* - allele absent, or not detected.

Below we develop the theory to predict the sustained impacts of many generations of small population size.

Heterozygosity decays exponentially over generations at a rate that is inversely proportional to effective population size ( $N_e$ )

Equation 8.2 can be extended to obtain an expression for the effects of long-term constant population size on heterozygosity, as follows:

$$H_1 = \left(1 - \frac{1}{2N_e}\right)H_0$$

and

$$H_2 = \left(1 - \frac{1}{2N_e}\right)H_1$$

By substituting the initial expression for  $H_1$  in the second equation

$$H_2 = \left(1 - \frac{1}{2N_e}\right)^2 H_0$$

and, by extension, the heterozygosity at generation  $t$  becomes,

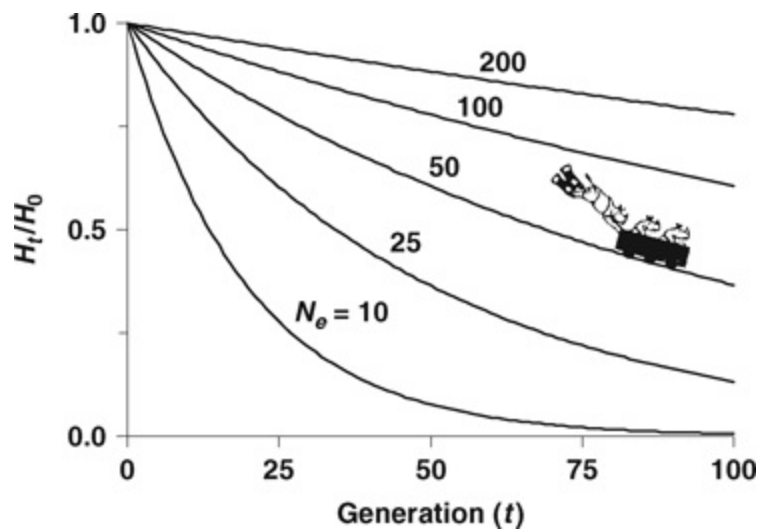
$$H_t = \left(1 - \frac{1}{2N_e}\right)^t H_0$$

This is usually expressed as the proportion of the initial heterozygosity remaining at generation  $t$ :

$$\frac{H_t}{H_0} = \left(1 - \frac{1}{2N_e}\right)^t \sim e^{-t/2N_e} \quad (11.1)$$

Predicted exponential declines in heterozygosity with generation number, in different sized populations, are shown in [Fig. 11.1](#). The important points of this relationship are:

- heterozygosity is lost at a rate that depends upon the effective population size, rather than the census size
- timescale of loss depends on generations, not years
- loss of heterozygosity continues with generations, in an exponential decay process
- half of the initial heterozygosity is lost in  $1.4N_e$  generations.



**Fig. 11.1** Predicted decline in heterozygosity over generations in different sized populations (after Foose 1986).

Example 11.1 illustrates the use of [Equation 11.1](#) to predict the loss of heterozygosity over 50 generations in populations with  $N_e$  of 500 versus 25. The former lose only about 5% of their initial heterozygosity over 50 generations, while populations with  $N_e = 25$  lose 64% of their initial heterozygosity.

### Example 11.1 Expected loss of heterozygosity due to sustained population size in large and small populations

The expected proportion of heterozygosity retained over 50 generations in a population of effective size 500 from [Equation 11.1](#) is:

$$\frac{H_t}{H_0} = \left(1 - \frac{1}{2N_e}\right)^t = \left(1 - \frac{1}{2 \times 500}\right)^{50} = \left(\frac{999}{1000}\right)^{50} = 0.951$$

i.e. this large population will lose only about 5% of its initial heterozygosity in 50 generations.

For a small population with  $N_e = 25$ , the proportion of initial

heterozygosity retained at generation 50 is expected to be

$$\frac{H_t}{H_0} = \left(1 - \frac{1}{2N_e}\right)^t = \left(1 - \frac{1}{2 \times 25}\right)^{50} = \left(\frac{49}{50}\right)^{50} = 0.364$$

Consequently, this small population will lose 64% of its initial heterozygosity in 50 generations.

---

The shorter the generation length, the more rapid will be the loss per year. Consequently, similar sized populations of black-footed ferrets with a generation length of two years will lose genetic diversity more rapidly than elephants with a generation length of 26 years.

To apply this theory we need to know effective population sizes. Lynch (2006) has estimated that effective size average  $10^8$  for prokaryotes,  $10^7$  for unicellular eukaryotes,  $10^6$  for invertebrates,  $10^4$  for vertebrates,  $10^6$  for annual plants and  $10^4$  for trees. These averages are typical of non-threatened species, but  $N_e$  estimates are often far smaller for threatened species. Details of estimating  $N_e$  for specific species are given later in this chapter.

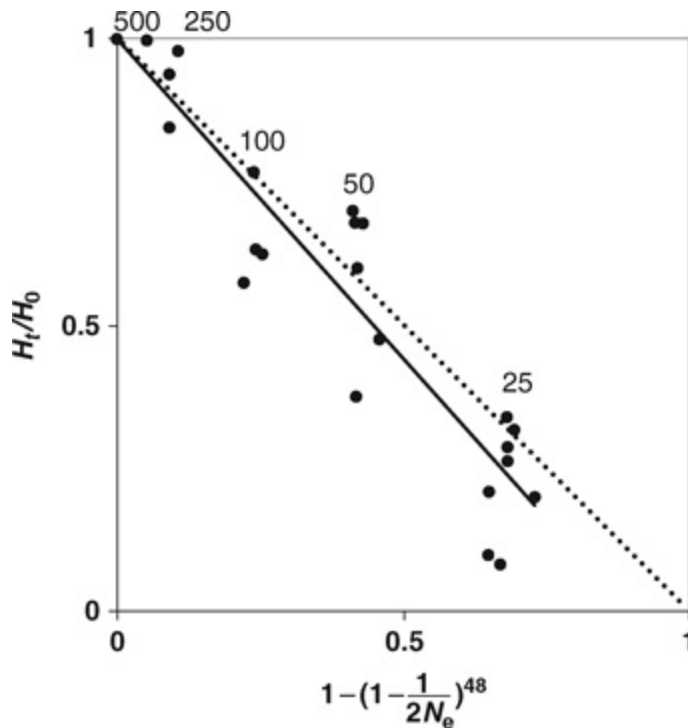
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In small populations, heterozygosity for microsatellite loci is lost at approximately the rate predicted by simple neutral theory

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In developing this theory, we have assumed that alleles are neutral and that their loci are unlinked – assumptions that may not apply in practice. Microsatellite variation is indeed lost approximately as described by [Equation](#)

11.1 (Fig. 11.2), while allozyme alleles and quantitative genetic variation are lost at a somewhat slower rate (Montgomery *et al.* 2000, 2008; Gilligan *et al.* 2005). Genetic diversity is lower in mammal populations that have experienced demographic threats than in those that have not (Garner *et al.* 2005).



**Fig. 11.2** Comparisons of observed and predicted proportion of initial microsatellite heterozygosities retained after 48 generations ( $H_{48}/H_0$ ) in captive fruit fly populations of different size plotted against  $1 - (1 - 1/[2N_e])^{48}$  (after Montgomery *et al.* 2009). Numbers at the top are effective population sizes. The solid line indicates the regression line of best fit and the dotted line the neutral prediction. *Genetic diversity is lost at approximately the predicted rate.*

Equation 11.1 predicts the expected (i.e. average) fate of heterozygosity, but the behaviour of individual loci can be highly variable due to the stochastic properties involved, especially in small populations. Heterozygosity estimates derived from the average of several loci yield results closer to predictions than those based on a single locus. Likewise, variation among replicate populations is expected to be greater for smaller

populations than for larger populations, as described in [Chapter 14](#).

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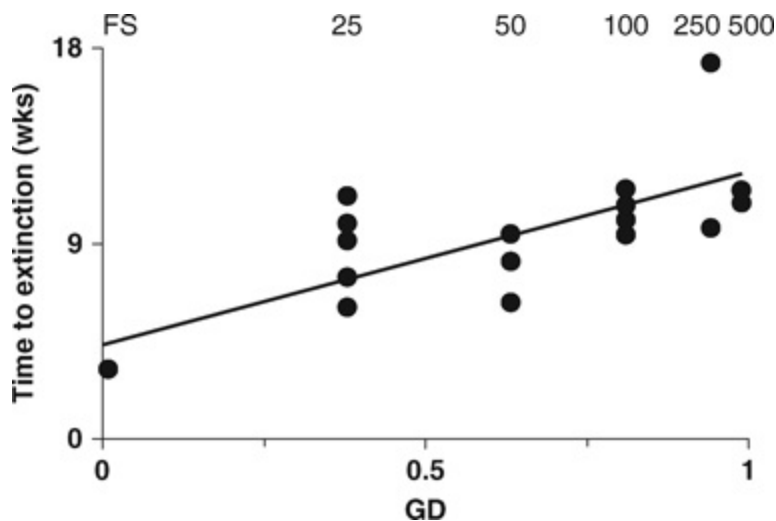
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Small populations lose genetic variation for reproductive fitness

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The equations for neutral loci do not apply to genetic variation for reproductive fitness, as it is subject to natural selection. However, we argued in [Chapter 9](#) that such genetic diversity would also be lost in small populations and this has been confirmed in an experimental test with fruit flies ([Fig. 11.3](#)).



**Fig. 11.3** Loss of evolutionary potential in small fruit fly populations (R. Frankham, E. H. Lowe, L. M. Woodworth, M. E. Montgomery & D. A. Briscoe, unpublished data). Populations founded from the same source population were maintained at different effective sizes (numbers at top of figure) for 50 generations. Equal numbers were used to establish large cage populations that were subjected to increasing concentrations of NaCl. The weeks to extinction are plotted against GD, proportion of neutral genetic diversity maintained =  $(1 - 1/[2N_e])^{50}$ . Numbers at the top are effective

population sizes and FS refers to populations full-sib mated for 35 generations. *Extinctions occur sooner, on average, in populations with less genetic diversity.*

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When populations fluctuate in size over generations, loss of genetic diversity is most strongly influenced by the minimum size

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So far our theory has only considered populations with constant sizes. However, most real populations fluctuate in size from generation to generation. Familiar examples are ‘plague species’, such as locusts and domestic mice. Such fluctuations have profound influences on heterozygosity (discussed here), on effective population size (see below) and on inbreeding ([Chapter 12](#)). The expression for the effect of varying population sizes on heterozygosity is obtained as the product ( $\Pi$ ) of the proportions of heterozygosity retained in each of the generations, as follows:

$$\frac{H_t}{H_0} = \prod_{i=1}^t \left(1 - \frac{1}{2N_{ei}}\right) \quad (11.2)$$

Reductions in heterozygosity are most strongly dependent on the generation with the smallest effective population size. For example, a population with effective sizes of 10, 100, 1000 and 10 000 over four generations loses 5.5% of its heterozygosity (Example 11.2), with almost all of the loss (5%) due to the generation with  $N_e = 10$ .

---

### **Example 11.2 Loss of heterozygosity with fluctuating**



## population sizes

The expected proportion of heterozygosity retained in a population with effective sizes of 10, 100, 1000 and 10 000 over four generations, is determined using [Equation 11.2](#), as follows:

$$\begin{aligned}\frac{H_t}{H_0} &= \prod_{i=1}^t \left(1 - \frac{1}{2N_{ei}}\right) \\ &= \left(1 - \frac{1}{20}\right) \left(1 - \frac{1}{200}\right) \left(1 - \frac{1}{2000}\right) \left(1 - \frac{1}{20\,000}\right) \\ &= 0.95 \times 0.995 \times 0.9995 \times 0.99995 = 0.945\end{aligned}$$

Consequently, the population loses 5.5% of its heterozygosity over the four generations, the great majority (5%) being due to the generation of size 10.

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## Relationship between population size and genetic diversity in wild populations

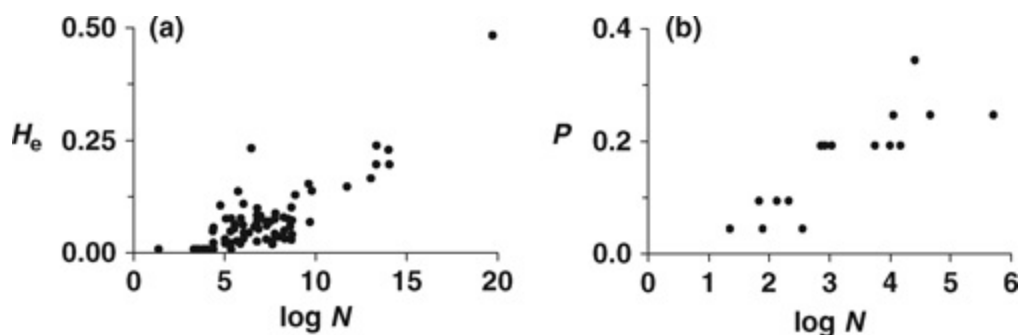
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Levels of genetic diversity are related to population size, both across species and among populations within species

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Positive correlations between population size and genetic diversity in wild populations are expected, based on [Equations 11.1](#) and [11.2](#). This assumes that most genetic diversity is neutral in small populations or, at most, weakly selected and subject to genetic drift ([Chapter 9](#)), and that current population sizes reflect historic effective population sizes.

There is overwhelming evidence from meta-analyses for associations between population size and nuclear genetic diversity, both within and across species (Hamrick & Godt 1989; Frankham 1996; Leimu *et al.* 2006). For example, a strong relationship has been found between genetic diversity and population size across a wide array of species (Fig. 11.4a), with population size explaining around one-half of the variation in heterozygosity among species. An example of the relationship within species is shown in Fig. 11.4b. Further, geographic range, a correlate of population size, is the major factor explaining levels of genetic diversity within plant species (Hamrick & Godt 1989). Not all studies show significant relationships between population size and genetic diversity. Some of these have insufficient statistical power while, in others, current population size does not reflect historic effective population size.



**Fig. 11.4** Relationship between genetic diversity and population size among and within species. (a)  $H_e$  versus logarithm of population size across species (after Frankham 1996), and (b) per cent polymorphism versus population size among populations within the New Zealand conifer *Halocharpus bidwilli* (after Billington 1991).

There are conflicting reports about the relationship between mtDNA diversity and population size across species, with different mtDNA mutation rates in different major taxa and frequent selective sweeps being possible explanations (Frankham 1996; Bazin *et al.* 2006; Lynch *et al.* 2006; Mulligan *et al.* 2006).

Endangered species typically have lower genetic diversity than related non-endangered species

Endangered species, by definition, have small or declining population sizes, and are therefore expected to have lower genetic diversity than non-endangered species. On average, endangered species have only about 65% of the molecular heterozygosity of taxonomically related, non-endangered species (Spielman *et al.* 2004a; see Table 3.5). For example, the critically endangered northern hairy-nosed wombat from Australia, with a population size of only ~100, has less genetic diversity than its non-endangered southern relative (Box 11.3).

**Box 11.3 Low genetic diversity in the critically endangered northern hairy-nosed wombat (Taylor et al. 1994; Beheregaray et al. 2000)**

The northern hairy-nosed wombat has had a low population size for a considerable period of time and now exists as a single population of approximately 100 individuals in central Queensland, Australia (see chapter front for locations). The population has lower levels of microsatellite genetic diversity than its nearest relative, the southern hairy-nosed wombat, which exists in larger numbers further south in the continent.

Species	<i>A</i>	<i>H<sub>e</sub></i>
Northern	2.1	0.32
Southern	5.9	0.71

## Loss of genetic diversity for haploid, sex-linked and polyploid loci

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Loss of genetic diversity due to genetic drift in populations of the same size is fastest for mtDNA and chloroplast DNA loci, followed in order by haploids, sex-linked loci, diploids and tetraploids

Species differ in the number of chromosomal sets they contain, from haploid ( $n$ ), through diploid ( $2n$ ), to polyploid (triploid –  $3n$ , tetraploid –  $4n$ , etc.). Sex-linked (X or Z) and Y- and W-linked loci have different numbers in the two sexes. Further, chloroplast and mitochondrial genomes are typically maternally transmitted. Since loss of genetic diversity is a sampling process, the rate of loss depends on the number of gene copies transmitted from parents to offspring (Bever & Felber 1994) (Table 11.1). There is an inverse relationship between loss of genetic diversity and number of gene copies transmitted each generation, as illustrated in Example 11.3. For example, Y chromosome loci and mtDNA are expected to lose genetic diversity at four times the rate of autosomal loci, while tetraploids lose it at half the rate of diploid.

**Table 11.1 Rates of loss of expected heterozygosity ( $H_e$ ) per generation in populations of the same size for neutral loci that are haploid, sex-linked, Y-linked, diploid, tetraploid, or in mtDNA or chloroplast DNA.  $N_e$  is effective population size and  $N_{ef}$  and  $N_{em}$  are the effective numbers of females and males, respectively. It is assumed that only one mtDNA or chloroplast genome per gamete contributes to the next generation and that we are dealing with idealized populations (Wright 1969)**

Mode of inheritance	Rates of loss of genetic diversity ( $H_e$ )
Haploid	$\frac{1}{N_e}$
Sex-linked (or haplo-diploid)	$\frac{1}{1.5N_e}$
Y-linked	$\frac{1}{N_{em}}$
Diploid	$\frac{1}{2N_e}$
Tetraploid	$\frac{1}{4N_e}$
Chloroplast DNA	$\frac{1}{N_{ef}}$
mtDNA	$\frac{1}{N_{ef}}$

Observed genetic diversity accords with these predictions, generally being lowest for Y or W chromosome loci, intermediate for X loci and highest for autosomal loci (Hedrick & Parker 1997; Berlin & Ellegren 2004; Handley *et al.* 2006). Further, genetic diversity is typically higher in tetraploid than diploid knapweed (Brown & Young 2000; Hardy & Vekemans 2001; Luttikhuisen *et al.* 2007). Thus, concerns about reductions in evolutionary potential are less for polyploids than for diploids (Chapter 17).

### Example 11.3 Loss of genetic diversity for haploid, sex-linked, diploid, tetraploid and mtDNA loci in a small population

Rates of loss of genetic diversity (Hardy–Weinberg heterozygosity) in a population composed of only five male and five female breeders are:

$$\text{Haploid loss} = \frac{1}{N_e} = \frac{1}{10}$$

$$\text{Sex-linked loss} = \frac{1}{1.5N_e} = \frac{1}{15} = 0.067$$

$$\text{Y-linked loss} = \frac{1}{N_{em}} = \frac{1}{5}$$

$$\text{Diploid loss} = \frac{1}{2N_a} = \frac{1}{20} = 0.05$$

$$\text{Tetraploid loss} = \frac{1}{4N_e} = \frac{1}{40} = 0.025$$

$$\text{mtDNA loss} = \frac{1}{N_{ef}} = \frac{1}{5}$$

Thus, the rates of loss of genetic diversity compared to the diploid case are four times for mtDNA, cpDNA and Y-linked loci (assuming equal sex-ratio), double for haploids, 33% greater for sex-linked, and half for tetraploids.

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## Effective population size

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The effective size of a population is usually less than the number of breeding adults as real populations deviate from the assumptions of the idealized population in constancy of population size, distribution of family sizes and sex-ratio

As all of the adverse genetic consequences of small populations depend on the effective population size, it is fundamentally important to have a clear understanding of the concept of effective population size and how it differs from census size.

We defined effective population size in [Chapter 8](#) as *the size of an ideal population that loses heterozygosity (or becomes inbred or drifts) at the same*

*rate as the observed population.* Any characteristic of a real population that deviates from the characteristics of an ideal population ([Chapter 8](#)) will cause the census size to be different from  $N_e$ . The primary factors are fluctuating population sizes over generations, high variance in family sizes and unequal sex-ratios.

Below we review evidence on  $N_e/N$  ratios and conclude that they are usually much less than 1. Later we consider the factors influencing  $N_e$  and examine their impacts, followed by the means for measuring  $N_e$  in real populations.

## **$N_e/N$ ratios**

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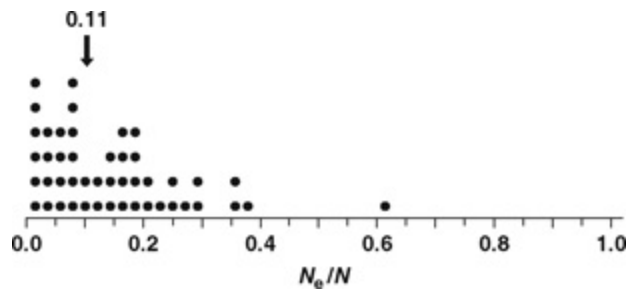
Estimates of  $N_e/N$  ratio that encompass all relevant factors average only 11% of census sizes

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The census population size ( $N$ ) is usually the only information available for most threatened species. Thus we need to know the ratio of effective to census size ( $N_e/N$ ), to infer  $N_e$ .

Values of  $N_e/N$  that include all relevant factors (comprehensive estimates) average only 11%, based on a meta-analysis of 38 cases ([Fig. 11.5](#)). Thus, long-term effective population sizes are substantially lower than census sizes. For example, the threatened winter run of chinook salmon in the Sacramento River of California has about 2000 adults, but its effective size was estimated to be only 85 ( $N_e/N = 0.04$ ), much lower than previously recognized (Bartley *et al.* [1992](#)). Genetic concerns are much more immediate with an effective

size of 85 than with 2000.



**Fig. 11.5** Distribution of effective/actual population size ( $N_e/N$ ) ratios. The estimates include the effects of fluctuations in population size, variance in family sizes and unequal sex-ratios, and thus reflect long-term effective population sizes (after Frankham 1995c). *The mean of estimates (arrow) is only 11%.*

The sobering implication is that long-term effective population sizes are only about 1/10 of actual sizes. Thus, endangered species with 250 adults have effective population sizes of about 25, and will lose half of their current heterozygosity for neutral loci in 34 generations, while threatened populations with  $N = 1000$  will have  $N_e$  of about 100 and lose half of their heterozygosity in 138 generations.

Recent evidence indicates that  $N_e/N$  ratios in highly fecund species such as fish, oysters and shrimp may be only  $\sim 10^{-3}$  to  $10^{-6}$  (Hauser *et al.* 2002; Turner *et al.* 2002; Hutchinson *et al.* 2003; Hedrick 2005b; Hoarau *et al.* 2005; Poulsen *et al.* 2006; Ovenden *et al.* 2007).

There has been discussion of what  $N$  to use in the divisor, whether average  $N$  or harmonic mean  $N$  (Kalinowski & Waples 2002). Since most information on  $N$  over time is reported as means, use of average  $N$  in  $N_e/N$  ratios is the most useful measure in practice (Frankham 1995c).

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Fluctuations in population size have greatest impact on reducing  $N_e$ , followed by variation in family sizes

---

The most important factor reducing the  $N_e/N$  ratio is fluctuation in population size, followed by high variation in family size (see below), with variation in sex-ratio having a smaller effect (Frankham [1995c](#)). Overlapping versus non-overlapping generations have no significant effect, nor do life history attributes. There were no clear or consistent differences in the ratio between major taxonomic groups, but such differences may well emerge in analyses including more recent data.

## Measuring effective population size

Since  $N_e \ll N$  we need to predict and measure the impacts on the effective population size of fluctuations in population size over generations, variation in family size, unequal sex-ratio and overlapping generations. These impacts are described below (see Crow & Kimura [1970](#) for derivations).

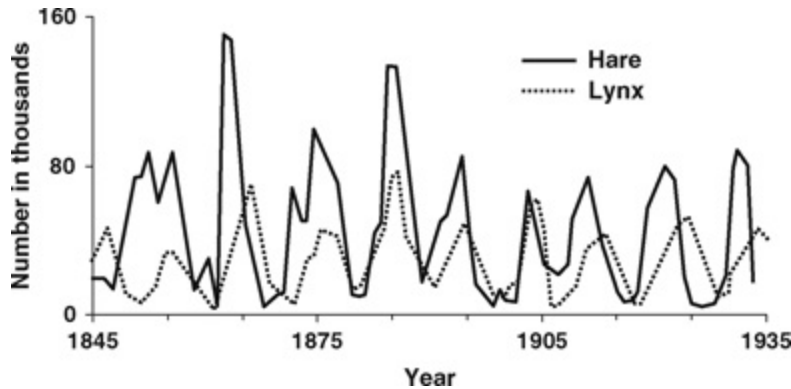
## Fluctuations in population size

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Fluctuations in population size over generations reduce  $N_e$  below the average number of adults

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Wild populations vary in numbers as a consequence of variation in food availability, climatic conditions, disease epidemics, catastrophes, predation, etc. (Reed *et al.* 2003b). For example, lynx and snowshoe hare populations fluctuate in size, with hares showing about a 30-fold difference between high and low years, and lynx about an 80-fold difference (Fig. 11.6).



**Fig. 11.6** Fluctuations in population size in lynx and snowshoe hares, based on pelt records of the Hudson Bay Company (after Hedrick 1983).

The effective size in a fluctuating population is not the arithmetic average, but the **harmonic mean** of the effective population sizes over  $t$  generations:

$$N_e \sim \frac{t}{\sum (1/N_{ei})} \quad (11.3)$$

where  $N_{ei}$  is the effective size in the  $i$ th generation and  $N_e$  the long-term, overall effective population size.

The long-term effective size is closest to the smallest single generation  $N_e$ . For example, the northern elephant seal was reduced to 20–30 individuals, but has since recovered to over 175 000. Its effective population size over this time is about 60 (Example 11.4). This is far closer to the minimum population size than to the average or the maximum. This relationship can best be explained by noting that an allele lost in a generation of low population size is not regained when the population size rises. Similarly, the inbreeding effects of small population size remain when a population

increases in size.

The predicted effects of fluctuations in population size on loss of genetic diversity, inbreeding and reproductive fitness have been verified in experiments with fruit flies (Woodworth *et al.* 1994). Fluctuations in population size reduce  $N_e$  in wild populations by an average of 65% (Frankham 1995c).



Lynx

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### Example 11.4 Reduction in $N_e$ due to fluctuations in population size

For simplicity, we assume that the northern elephant seal population declined from 175 000 to 20 and recovered to 175 000 over three generations and that these were the effective sizes for each of the generations. The effective size of the population is:

$$N_e \sim \frac{t}{[(1/N_{e1}) + (1/N_{e2}) + (1/N_{e3})]}$$
$$\sim \frac{3}{[(1/175\,000) + (1/20) + (1/175\,000)]} \sim 60$$

The effective size of 60 is much closer to the minimum size than the mean size (116 673) over the three generations.

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## Variation in family size

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When variation in family size exceeds that of the Poisson distribution, the  $N_e/N$  ratio is less than 1

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Family sizes (lifetime production of offspring per individual) in wild populations typically show greater variation than that expected (Poisson) for the idealized population, resulting in  $N_e < N$ . In a stable population of a randomly breeding species, the mean family size ( $k$ ) is an average of one for males and one for females. With the Poisson distribution, the proportions of families with 0, 1, 2, 3, 4 and 5 offspring are 0.135, 0.271, 0.271, 0.180, 0.090 and 0.036, respectively. The variance ( $V_k$ ) equals the mean for a Poisson distribution, so  $V_k / k = 1$  for a population with an idealized structure.

Most species have  $V_k/k$  ratios in excess of the value of 1 assumed for the idealized population ([Table 11.2](#)). High variation in family sizes in wildlife is due both to higher than expected number of individuals that contribute no offspring to the next generation (Hedrick [2005b](#)), and to very large and very small families. Where progeny of many families do not survive at all, the  $N_e/N$  ratio will approximate the proportion of families that survive and this may be very low in fish and shrimp ([Waples 2002](#)).

**Table 11.2   Variance in lifetime reproductive success ( $V_k$ ) and mean family size ( $k$ ) for a range of species, many threatened. As the ratio of  $V_k / k$  is greater than 1, variance is greater than for the Poisson distribution and effective size will be less than census size**

Species	Captive (c) or wild (w)	Sex	$V_k$	$k$	$V_k/k$	Reference
Mammals						
Asiatic lion	c	m	31.10	1.64	19.0	1
	c	f	34.00	1.67	20.4	
Eastern barred bandicoot	w	f+m	11.6	1.0	11.6	2
Golden lion tamarin	c	m	12.10	1.7	7.1	3
	c	f	13.5	1.6	8.4	
Golden-headed lion tamarin	c	m	7.27	1.07	6.8	1
	c	f	5.74	1.05	5.5	
Grevy's zebra	c	m	34.00	1.90	17.9	1
	c	f	1.20	1.14	1.1	
Przewalski's horse	c	m	23.44	1.18	19.9	1
	c	f	9.92	1.27	7.8	
Scimitar-horned oryx	c	m	127.90	1.48	86.4	1
	c	f	10.44	1.24	8.4	
Sumatran tiger	c	m	22.50	2.46	9.1	4
		f	16.61	2.09	7.9	
Birds						
Darwin's cactus finch	w	f+m	6.74	1.8	3.7	5
Darwin's large cactus finch	w	f+m	0.53	0.3	1.8	6
Darwin's medium ground finch	w	f+m	7.12	1.6	4.5	5
Pink pigeon	c	m	31.24	1.54	20.3	1
	c	f	5.74	1.05	5.5	1
Red-crowned crane	c	m	9.10	1.76	5.2	1
	c	f	4.80	1.64	2.9	1

*References:* 1, Dobson *et al.* (1992); 2, Sherwin & Brown (1990); 3, Ballou & Foose (1996); 4, Ballou & Seidensticker (1987); 5, Grant & Grant (1992); 6, Grant & Grant (1989).

The effect on  $N_e$  of variation in family sizes in a population otherwise having the structure of an idealized population is

$$N_e = \frac{4N - 2}{V_k + 2} \quad (11.4)$$

This is the single-generation effective population size due to family size alone. In the idealized population,  $V_k = 2$  for female and males combined, ( $V_k = 1$  for females and  $V_k = 1$  for males) so that  $N_e \sim N$ . With high variance in family size, the effective population size is reduced below  $N$ . For example, in

Darwin's cactus finch high variance in family size (6.74), compared to the Poisson expectation (2) reduces the effective population size to less than half the number of breeding individuals (Example 11.5). Over a range of species, variation in family sizes reduced average  $N_e$  to 54% of  $N$  (Frankham 1995c).

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### **Example 11.5 Reduction in effective population size through high variance in family size in Darwin's large cactus finch**

The variance in family sizes for Darwin's cactus finch is 6.74, compared to the value of 2 assumed for an idealized population (Table 11.2). Equation 11.4 can be rearranged to give

$$\frac{N_e}{N} \sim \frac{4}{V_k + 2}$$



Darwin's large cactus finch

Upon inserting the observed value into this equation, we obtain

$$\frac{N_e}{N} \sim \frac{4}{6.74 + 2} = 0.46$$

---

If a population varies in size from generation to generation, the equation describing the influence of variation in family size on effective size becomes:

$$N_e = \frac{Nk - 1}{k - 1 + (V_k/k)} \quad (11.5)$$

where  $N$  is the number of adults in the previous generations and  $k$  is the mean family size (again this is a single-generation  $N_e$ ). All other factors are assumed to conform to those of an idealized population. Example 11.6 illustrates the use of this equation for a captive Asiatic lion population; variation in family sizes results in a 92% reduction in effective size compared to the actual size.

### Example 11.6 Reduction in effective population size in Asiatic lions due to high variation in family sizes

Asiatic lions in captivity have an average family size of 1.65 and a variance in family size of 32.65 (Table 11.2). We rearrange Equation 11.5 to give an expression for  $N_e/N$ , by dividing both sides of the equation by  $N$ :

$$\frac{N_e}{N} = \frac{(Nk - 1)}{N[k - 1 + (V_k/k)]} \sim \frac{k}{[k - 1 + (V_k/K)]}$$



Asiatic lion

Substitution of the observed values into this equation gives

$$\frac{N_e}{N} \sim \frac{1.65}{[1.65 - 1 + (32.65/1.65)]} = 0.081$$

Thus, variation in family size reduces the effective size of the Asiatic lion population to 8% of the number of adults.

Equalization of family sizes (EFS) leads to an approximate doubling of the effective population size, compared to the actual size of the population

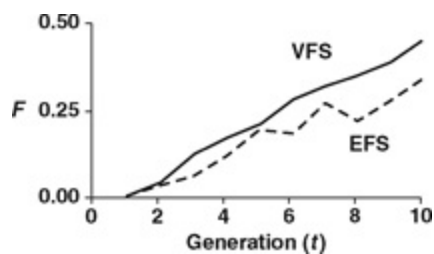
If variance in family size is less than that in the idealized population, we have the surprising result that  $N_e > N$ . In fact, if all individuals contribute equally to the next generation ( $V_k = 0$ ),  $N_e \sim 2N$ . This can be understood by recalling that the idealized population assumes that there is Poisson variance in family size. When all families contribute alleles equally to next generation, there is minimal distortion in allele frequencies and the proportion of the genetic diversity passed on is maximized. In a stable population of a monogamous species  $V_k = 0$  corresponds to each family contributing 1 female and 1 male to the next generation. This also allows inbreeding to be minimized ([Chapter 12](#)).

This observation is of critical importance in the management of captive populations. Equalization of family sizes (EFS) potentially allows limited breeding spaces for endangered species to be effectively doubled. The benefits of equalizing family sizes in minimizing loss of genetic diversity and inbreeding have been verified in experiments with fruit flies ([Box 11.4](#)). A variation on EFS is recommended for management of captive populations of endangered species ([Chapter 19](#)).



### Box 11.4 Experimental evaluation of the effects of variable versus equal family sizes on loss of genetic diversity, inbreeding, and reproductive fitness, using fruit flies (Borlase et al. 1993)

Twenty replicate populations were founded from a large population of wild fruit flies, and maintained for 10 generations. Ten replicates were managed with equal family sizes (EFS), and 10 with variable family sizes (VFS). Four female and four male parents were used in each generation ( $N = 8$ ). The effective population sizes for the two treatments were expected to be 16 for EFS and 8 for VFS, based on [Equation 11.4](#). Consequently, the EFS treatment is expected to lose less genetic diversity, have lower inbreeding and experience less reduction in reproductive fitness than the VFS treatment.

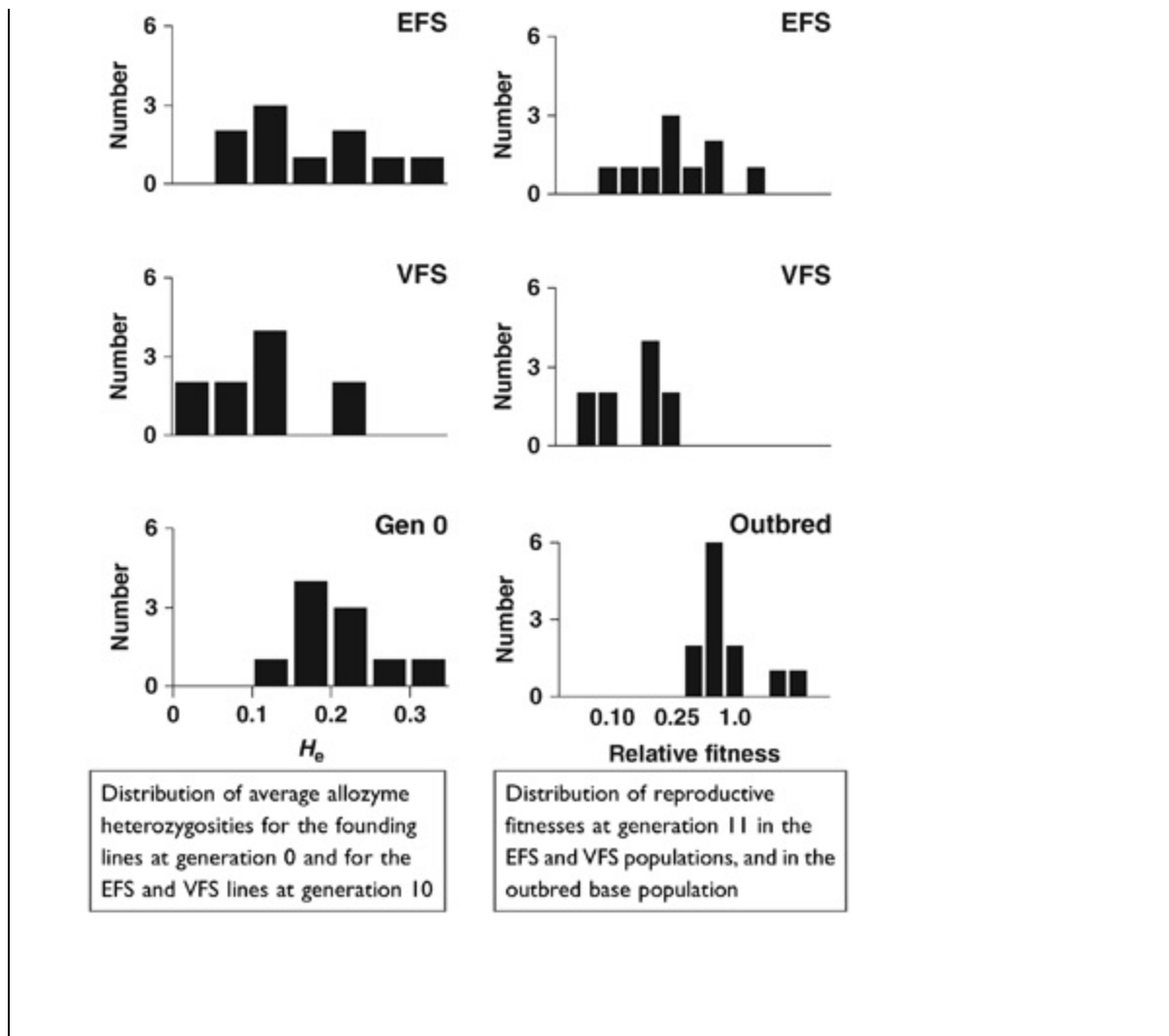


Levels of inbreeding in the EFS and VFS treatments

Each of these predictions was verified, as shown for allozyme heterozygosity (below left), inbreeding levels (margin), and reproductive fitness (below right). Quantitative genetic variation was also higher in EFS than in VFS populations (Frankham 2000a).



Fruit fly



## Unequal sex-ratio

Unequal sex-ratios reduce the effective size of populations to below that of a population with an equal sex-ratio

Sex-ratios among breeders often deviate from equality in wild populations.

There is typically an excess of females in mammals and a deficiency in birds (Donald 2007). Many mammals have harems (**polygyny**) where one male mates with many females, while many other males make no genetic contribution to the next generation. For example, a single male elephant seal may have a hundred or more females in his harem. In a few species, such as jacana birds, the situation is reversed (**polyandry**). Adult sex ratios in birds are frequently distorted, averaging around a 33% excess of males, and the situation is worse in threatened species (Donald 2007). The equation accounting for the effects of unequal sex-ratio is

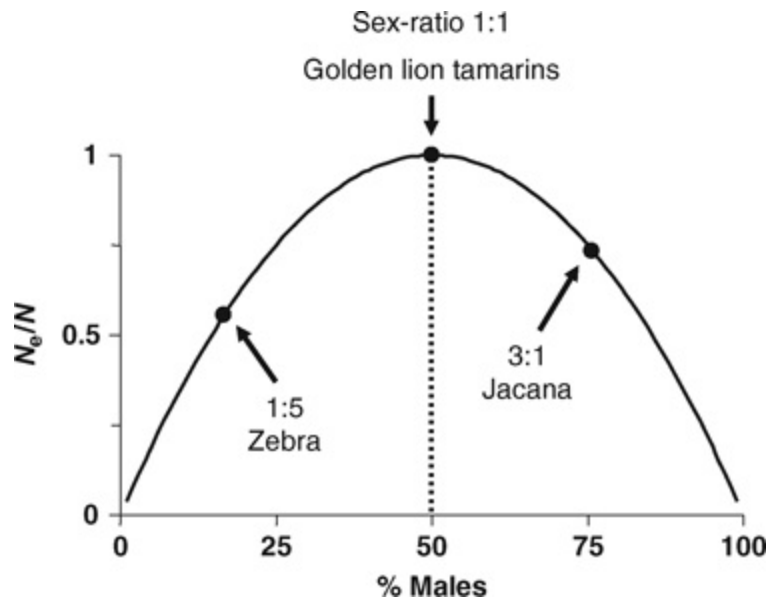
$$N_e = \frac{4N_{ef}N_{em}}{N_{ef} + N_{em}} \text{ (approx.)} \quad (11.6)$$

where  $N_{ef}$  is the effective number of breeding females and  $N_{em}$  is the effective number of breeding male parents. This is the single-generation effective population size due to this factor alone, with all other characteristics assumed to be those of an idealized population.

As the sex-ratio deviates from 1 : 1 in either direction, the  $N_e/N$  ratio declines (Fig. 11.7). For example, an elephant seal harem with one male and 100 females has an effective size of only 4 (Example 11.7).



Jacana



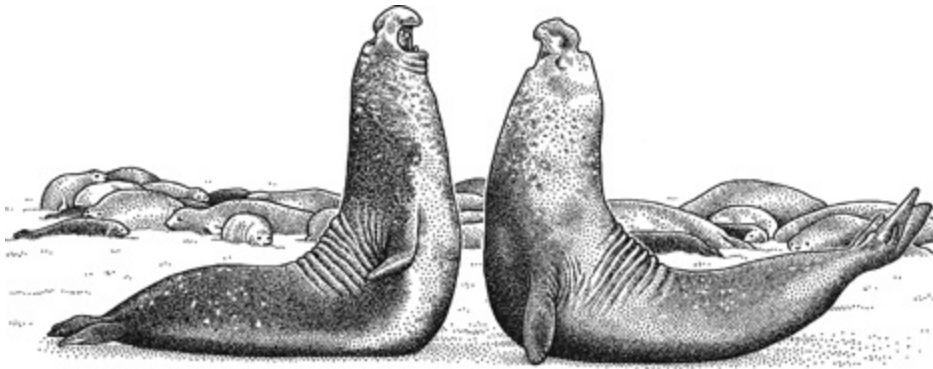
**Fig. 11.7** Effects of unequal sex-ratios on the  $N_e/N$  ratio. As the sex-ratio deviates from 1 : 1 in either direction the  $N_e/N$  ratio declines.

However, it is the lifetime sex-ratio over a generation that matters. While Southern elephant seal harems average about 40 females in any one breeding season (Jewell 1976), genetic data indicate that the sex-ratio over a generation is only about 5 females : 1 male (Slade *et al.* 1998). The predicted effects of unequal sex-ratio on loss of genetic diversity and inbreeding have been verified in experiments using fruit flies (Briton *et al.* 1994).

### Example 11.7 Reduction in effective size due to unequal sex-ratio in elephant seals

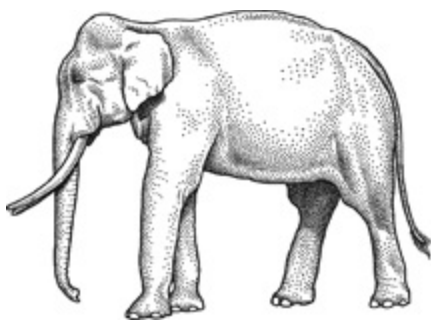
If a harem has one male and 100 females, the effective size is

$$N_e = \frac{4N_{ef}N_{em}}{N_{ef} + N_{em}} = \frac{4 \times 100 \times 1}{100 + 1} = 3.96$$



Thus the effective size of the harem is approximately 4% of the actual size (101).

Sex-ratios may also be distorted by hunting. For example, poaching of male Asian elephants for ivory in a southern Indian population resulted in an adult sex ratio of 605 females : 6 males (Sukumar *et al.* 1998). The population has an effective size of only 24 (Problem 11.2), and a  $N_e/N$  ratio of 0.04. Global warming may be distorting sex-ratios in turtles, crocodilians and other reptiles where sex is determined by incubation temperature (Hays *et al.* 2003).



Asiatic elephant

Overall, unequal sex-ratios have modest effects in reducing effective population sizes below actual sizes, resulting in an average reduction of 36% (Frankham 1995c).

## Exclusion of matings between close relatives

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Avoidance of selfing or sib-mating in random mating populations has little impact on  $N_e$

Self-fertilization is not possible in dioecious species, and many species avoid incestuous matings. However, these deviations from the assumptions of the idealized population have only minor impacts on loss of genetic diversity, and rates of inbreeding, as the probability of selfing or sib-mating is very low in random mating populations unless they are very small. The effects of these exclusions on the single-generation effective size (for populations otherwise behaving as idealized populations) are:

self-fertilization excluded

$$N_e = N + 1/2 \text{ (approx.)} \quad (11.7)$$

sib-mating also excluded

$$N_e = N + 2 \text{ (approx.)} \quad (11.8)$$

## Overlapping generations

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Overlapping generations do not have a consistent directional effect on  $N_e$

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Most natural populations have overlapping rather than the discrete generations assumed for idealized populations. The effects on  $N_e$  of overlapping generations are not clearly in one direction (Nunney 2002). For annual plants with a seed bank that survives for several years, the effective population size per generation is usually increased compared to the case with no seed bank, as seed banks generally reduce the fluctuations in  $N$  over generations. However, in polygamous animals where adults live and reproduce for several years, overlapping generations are more likely to reduce  $N_e$  compared to situations with non-overlapping generations. Dominant polygamous males may mate with more females over their lifetime and thus increase variation in family sizes. An empirical test for effects of overlapping generations on  $N_e/N$  ratios did not yield a significant effect, so overall impacts may be modest (Frankham 1995c).

Equations exist to evaluate the effects of overlapping generations on  $N_e$  (Nunney 2002; Engen *et al.* 2007), but they are rarely used in practical situations. Relatively complex computer models are more practical (Allendorf *et al.* 1991).

## Combinations of factors

Ultimately we wish to determine  $N_e$  resulting from the combined impact of all factors. Example 11.8 illustrates the combined impacts of variance in family sizes plus unequal sex-ratios for golden lion tamarins. Computations of the combined impacts of several factors on  $N_e$  are tedious and are usually done using computer software.

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**Example 11.8 Computing  $N_e$  in captive golden lion**

## tamarins due to the combined impacts of variance in family sizes and unequal sex-ratios (after Ballou & Foose 1996)

The numbers of female and male golden lion tamarins and the mean ( $k$ ) and variance ( $V_k$ ) of the offspring numbers they contributed to the next generation are:

	Females	Males
Adult numbers	275	269
$k$	1.6	1.7
$V_k$	13.5	12.1

The effective size in females ( $N_{ef}$ ) due to variation in family sizes using Equation 11.5 is

$$N_{ef} = \frac{N_f k - 1}{k - 1 + (V_k/k)} = \frac{275 \times 1.6 - 1}{1.6 - 1 + (13.5/1.6)} = 48.6$$

The effective size in males ( $N_{em}$ ) due to variation in family sizes is

$$N_{em} = \frac{N_m k - 1}{k - 1 + (V_k/k)} = \frac{269 \times 1.7 - 1}{1.7 - 1 + (12.1/1.7)} = 58.4$$

The overall  $N_e$  is obtained by taking into account the unequal sex-ratio, as follows:

$$N_e = \frac{4N_{ef}N_{em}}{N_{ef} + N_{em}} = \frac{4 \times 48.6 \times 58.4}{48.6 + 58.4} = 106.1$$

Thus, the effective size of the golden lion tamarin population is 106, while its actual size is 544, giving a  $N_e/N$  ratio of 0.2.

## Inbreeding and variance effective sizes



Different effective population sizes are required to predict inbreeding and loss of genetic diversity. Often these are very similar, but may differ when there are large changes in population size over generations

So far we have discussed effective population size as though it was a single parameter. However, there are three parameters: the inbreeding, eigenvalue and variance effective sizes. Strictly, the effective size determining loss of genetic diversity is the eigenvalue effective size. The inbreeding effective size determines the rate of inbreeding, and the variance effective size determines diversification among replicate populations. The three effective sizes have very similar values with constant population sizes but, in some circumstances, they can be quite different, especially when there are major changes in population size over generations. Further details are provided in Crow & Kimura (1970) and Templeton & Read (1994).

## Estimating $N_e$

Demographic and genetic methods are used in practical situations to estimate  $N_e$ . Demographic methods are based on Equations 11.3–11.8 above. They require extensive demographic data that are often unavailable.

Genetic methods are based on equations relating  $N_e$  and:

- loss of heterozygosity over generations (Equation 11.1 and Example 11.9)
- changes in allele frequencies over time due to genetic drift (Tallmon *et al.* 2004a; Chapter 14)
- rate of decay in linkage disequilibrium among loci (Waples 2006), as has been used in chinook salmon and other fish by Bartley *et al.* (1992)

- heterozygosity excess due to drift in male and female frequencies (Wang 2005)
- rate of increase in pedigree inbreeding coefficient, or loss of pedigree gene diversity (Equation 12.5)
- loss of allelic diversity (Saccheri *et al.* 1999)
- drift–mutation equilibrium for DNA sequence or microsatellite data operating over long evolutionary times (Wang 2005)
- joint estimates of  $N_e$  and migration rate, for populations that are not closed (Wang & Whitlock 2003); other methods assume that populations are closed.

Example 11.9 illustrates the estimation of  $N_e$  from loss of genetic diversity over generations in the endangered northern hairy-nosed wombat. The effective size was approximately 7, compared to the actual size of about 70.

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**Example 11.9 Estimation of  $N_e$  from loss of genetic diversity over time in the critically endangered northern hairy-nosed wombat (Taylor *et al.* 1994).**

The northern hairy-nosed wombat declined over the last 120 years (~12 generations) from more than a thousand individuals to 25 in 1981 and had recovered to about 70 individuals by the early 1990s. It retained 41% of its heterozygosity over this period.

We estimate the long-term effective population size for this species using Equation 11.1, as follows:

$$\frac{H_t}{H_0} = \left(1 - \frac{1}{2N_e}\right)^t \sim e^{-t/2N_e} = e^{-12/2N_e} = 0.41$$

Taking  $\log_e$ , we obtain

$$\ln(0.41) = \frac{-12}{2N_e}$$

and by rearranging

$$N_e = \frac{-12}{2\ln(0.41)} = 6.7$$

Thus, the effective population size of the endangered northern hairy-nosed wombat over the last 120 years has been about 7.

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Long-term evolutionary effective population size can be determined from mutation–drift equilibrium theory if mutation rates are known

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Drift–mutation equilibrium can be used to estimate evolutionary effective size for females from mtDNA diversity, as follows (Hedrick [2005a](#)):

$$N_{ef} = \frac{\Theta}{2u} \quad (11.9)$$

where  $\Theta$  is the average proportion of nucleotide sites that differ between random pairs of haplotypes and  $u$ , the base substitution rate per generation.

For nuclear loci, the estimate of  $N_e$  is:

$$N_e = \frac{\Theta}{4u} \quad (11.10)$$

As shown in Example 11.10, the effective population size for gray whales

was determined as 39 117 using estimates of  $\Theta$  and the mutation rate.

Controversial work from Roman & Palumbi (2003) used this method, with adjustments for sex-ratio,  $N_e/N$  ratio and proportion of juveniles, to estimate that humpback and fin whales had pre-whaling numbers of 240 000 and 360 000 in the North Atlantic. These values are much greater than previous estimates of pre-whaling populations and 6–20 times higher than present population sizes. These results have critical implications in the debate about whether the numbers of these two species have recovered sufficiently to justify resumption of harvesting.

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### **Example 11.10 Estimating effective population size in gray whales using Equation 11.10**

Alter *et al.* (2007) reported an average  $\Theta$  per generation for seven autosomal loci of 0.001161, a substitution rate of  $4.79 \times 10^{-10}$  base pairs per year and an average generation length of 15.5 years. The substitution rate is presumed to reflect the mutation rate  $u$ , but has to be converted to mutation rate per generation by multiplying by the generation length.

$$u = 15.5 \times 4.79 \times 10^{-10} = 7.42 \times 10^{-9} \text{ mutations/generation}$$

Using Equation 11.10, the estimate of effective population size is

$$N_e = \frac{\Theta}{4u} = \frac{0.001161}{4 \times 7.42 \times 10^{-9}} = 39\,117$$

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A number of the estimators of  $N_e$  are biased and comparisons using computer simulations have found considerable differences in precision amongst estimates (Tallmon *et al.* 2004a; Waples 2006). Caballero (1994) reviews demographic estimation procedures and Wang (2005) reviews

genetic methods of estimating  $N_e$ .

## Gene trees and coalescence

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Gene trees and coalescence analyses allow more powerful genetic analyses, as they add a time dimension

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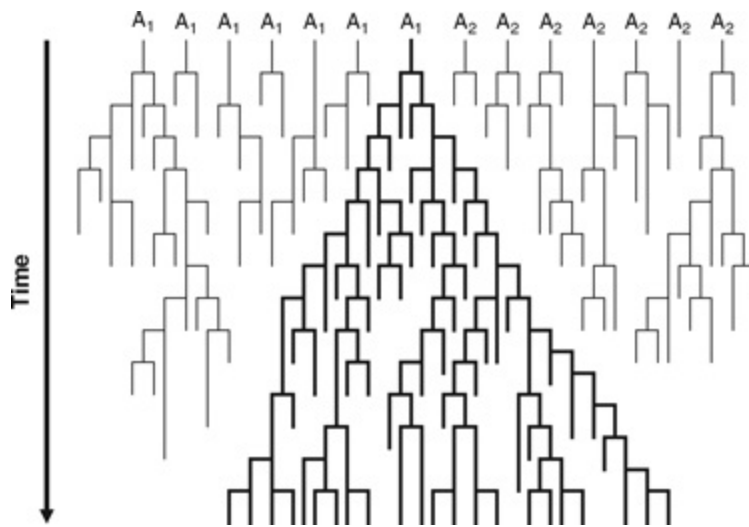
DNA sequences retain information on their prior evolutionary history of population sizes, population fragmentation, selection history, etc. For example, two alcohol dehydrogenase alleles in *Drosophila* that differ by two bases are more closely related and diverged more recently than two alleles that differ by 11 base pairs ([Fig. 3.2](#)). **Coalescence** and **gene trees** allow us to extract this information by working backwards in time (Rosenberg & Nordborg [2002](#)). Based on sampling theory for neutral alleles, they provide a null hypothesis against which to test data and to discriminate possible reasons for deviations. Consequently, they are more powerful than conventional analyses that use only current distributions and patterns of DNA sequence differences.

In the following sections we present the concept of gene trees and coalescence and show examples of how they are used to estimate effective population size, estimate evolutionary time in populations, and infer the occurrence of population bottlenecks. In [Chapter 21](#) we illustrate their applications to defining population structures, detecting migrants and detecting selection.

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Coalescence is the analysis of the distribution and differences among DNA sequences for alleles and the events and time frames involved in developing these differences

Coalescence is based on the concept that if we trace current allelic sequences in a population back long enough through time, they **coalesce** to a single individual sequence (Fig. 11.8). Other alleles, once present, have been lost by genetic drift or selection, and new alleles have been generated through mutation. The evolutionary pattern of the extant distribution of alleles at a locus can be represented as the branches of a tree coalescing back to a single ancestral allelic sequence.



**Fig. 11.8** Gene trees and coalescence: a possible history of descent of DNA sequences in a population that began at time 0 (top of figure) with 14 copies, representing two alleles. Some sequences leave one or more copies in the subsequent generation while others become extinct. Sequences present at the bottom are all descended from (coalesce to) a single ancestral copy of the  $A_1$  allele (this lineage is shown in the heavier black lines in the figure). If the failure of the ancestral sequences to leave descendants was random, the sequences at the bottom could equally well have come from any other

ancestral copy at generation 0 (after Futuyma [1998](#)).

Gene trees trace the evolutionary history of the alleles (e.g. mtDNA haplotypes) in the same manner as tracing the origin, or loss, of alleles through pedigrees, such as tracing the sex-linked haemophilia allele in the royal families of Europe back to Queen Victoria of Great Britain.

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Coalescence provides theory to model the survival and spread of alleles over time in the lineages of a population

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Neutral theory allows us to predict the time in generations back to coalescence. Under neutral theory, two alleles may descend from the same ancestral allele in the previous generation with a probability  $1/N_{ef}$  for mtDNA (or  $1/2N_e$  for a diploid locus). Alternatively, two alleles may derive from two different alleles in the previous generation (or derive from the same allele many generations ago) with probabilities  $1 - 1/N_{ef}$  (where  $N_{ef}$  is the effective number of females). Under this neutral model of genetic drift, the coalescence process takes a characteristic time. In a diploid population with  $k$  alleles at a neutral locus, the average time in generations ( $T_k$ ) back to the previous coalescent event when there were  $k - 1$  alleles, is (Hedrick [2005a](#)):

$$T_k = \frac{4N_e}{[k(k-1)]} \text{ generations} \quad (11.11)$$

Thus, the times during which there are 5, 4, 3 and 2 alleles are  $4N_e/20$ ,  $4N_e/12$ ,  $4N_e/6$  and  $4N_e/2$  generations, respectively. The time for all  $k$  alleles

in the population to coalesce back to one allele is  $4N_e [1 - (1 / k)]$  generations (Fig. 11.9). Example 11.11 illustrates the calculation of coalescence times in diploid populations with effective size 50 and 100. Note that the coalescence times increase in direct proportion to population size.

---

### **Example 11.11 Estimating coalescence times**

In a population of  $N_e = 50$  with three alleles, the expected time to its previous coalescence (when the population only had two alleles) is:

$$T_3 = \frac{4N_e}{[k(k-1)]} = \frac{(4 \times 50)}{(3 \times 2)} = 33 \text{ generations}$$

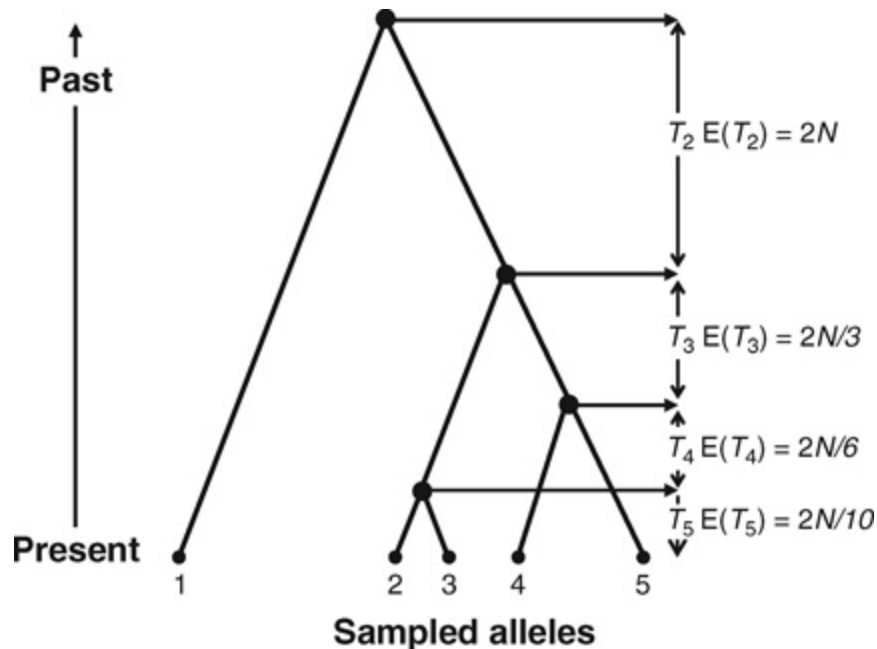
For  $N_e = 100$  coalescence takes:

$$T_3 = \frac{4N_e}{[k(k-1)]} = \frac{(4 \times 100)}{(3 \times 2)} = 67 \text{ generations}$$

Thus, the coalescence takes twice as long in a population of twice the size.

---





**Fig. 11.9** Gene trees and coalescence times: an example of a gene tree for five alleles. The large circles indicate coalescent events (after Hedrick 2000).  $T_i$  is the length of time it takes for the  $i$  alleles present to coalesce into  $i - 1$  alleles, and intervals are shown proportional to their expected times in generations.

## Summary

1. Genetic diversity decays over generations in small closed populations at a rate inversely dependent on the effective population size.
2. The effective population size is the number of individuals that would give rise to the observed loss of heterozygosity or to the calculated inbreeding coefficient if they behaved in the manner of the idealized population.
3.  $N_e$  is typically much less than adult population sizes, with long-term  $N_e$  averaging about 10% of the census sizes.
4. The effective population size is reduced by fluctuations in population size across generations, high variance in family size and by unequal sex-ratios.
5. Effective population sizes are measured either by using a series of demographic equations that account for the variables in 4, or by

using different genetic methods that evaluate changes in genetic diversity over time, or linkage disequilibrium in a population, etc.

6. Gene trees and coalescence are powerful tools for adding a time dimension to past evolutionary events, for detecting bottlenecks, population structure and immigration, as well as other aspects of a species' past.

## Further reading

Crow & Kimura (1970) *An Introduction to Population Genetics Theory*. Advanced treatment of effective population size and other topics in this chapter.

Falconer & Mackay (1996) *Introduction to Quantitative Genetics*. Clear treatment of effective population size and other topics in this chapter.

Frankham (1995c) A review and meta-analysis of  $N_e/N$  ratios and factors that affect the ratio.

Frankham (1996) Review and meta-analysis of evidence on the relationship between population size and genetic diversity.

Hedrick (2005a) *Genetics of Populations*. Has a clear introduction to coalescence and gene trees.

Lande & Barrowclough (1987) Conservation-orientated review of the issues in this chapter.

Rosenberg & Nordborg (2002) Concise review on coalescence and gene trees.

Saccheri & Hanski (2006) Fine review of the impact of natural selection on population dynamics.

## Software

EASYPOP: Free software for population genetics simulations (Balloux 2001). [www.unil.ch/izea/software/easypop/html](http://www.unil.ch/izea/software/easypop/html)

MLNE: Free software for simultaneous estimation of effective population size and migration rate from marker allele frequencies (Wang & Whitlock 2003). [www.zoo.cam.ac.uk/ioz/software.htm#MLNE/](http://www.zoo.cam.ac.uk/ioz/software.htm#MLNE/)

NeEstimator: Free software for estimating effective population size, using a variety of genetic methods (Peel *et al.* 2004). [www.dpi.qld.gov.au/cps/rde/xchg/dpi/hs.xsl/28\\_6908\\_ENA\\_Print.htm](http://www.dpi.qld.gov.au/cps/rde/xchg/dpi/hs.xsl/28_6908_ENA_Print.htm)

## Problems

- 11.1 Loss of heterozygosity in small populations. (a) What proportion of its initial genetic diversity will be retained after 100 years in the Javan rhinoceros which has a population size of 60 and a generation length of ~20 years? (b) What proportion of the initial heterozygosity will be retained if the effective population size is only 10?
- 11.2 Loss of heterozygosity. Use Equation 11.1 to determine the time taken (in units of  $N_e$  generations) for a population of size  $N_e$  to lose (a) 50% of its initial heterozygosity (set  $H_t/H_0 = 0.5 = e^{-t/2N_e}$ , then take  $\ln$  of both sides of the equation and rearrange), and (b) 95% of its initial heterozygosity.
- 11.3 Loss of heterozygosity with fluctuating population sizes. Compare the loss in heterozygosity in a population that fluctuates 100, 10, 100, 200 with one that fluctuates 200, 100, 100, 10.
- 11.4 Effective population size. What is the effective population size in a population of British field cricket if its size fluctuates 10, 100, 1000, 250 over generations?
- 11.5 Effective population size. What is the effective population size in a population of red-crowned cranes with four families that contribute 0, 1, 2 and 5 offspring to the next generation?
- 11.6 Effective population size. What is the effective size of the Asian elephant population in Periyar, southern India where poaching of males has resulted in an adult sex-ratio of 6 males to 605 females? What would the effective size be with a 'normal' adult elephant sex-ratio of 1 : 3 (202 males : 605 females)?

- 11.7** Effective population size. If the adult population of Sumatran tigers consists of 60 males and 80 females, use the data in [Table 11.2](#) to determine its  $N_e$  due to the combined effects of variance in family sizes and unequal sex-ratio.
- 11.8** Estimating  $N_e$  from loss of genetic diversity. Rearrange [Equation 11.1](#) and use it to estimate the effective size for the Mauritius kestrel, given that it has lost 57% of its heterozygosity in ~17 generations (Groombridge *et al.* 2000).
- 11.9** Estimating effective population size for females from mutation–drift equilibrium. Use [Equation 11.9](#) to determine  $N_{ef}$  for humpback whales, given that mtDNA  $\Theta$  is 0.0216, the neutral mutation rate for mtDNA is  $1.5 \times 10^{-8}$  per base per year and the generation length is 24 years (from Roman & Palumbi 2003).
- 11.10** Coalescence time. What is the mean time to complete coalescence for four alleles in a population of  $N_e = 50$ ? In a population of  $N_e = 10$ ?

## Practical exercises: Computer simulations

Use EASYPOP or an equivalent computer simulation package to complete the following:

1. Using initial frequencies of  $p = q = 0.5$ , run 50 replicate simulations with population sizes of 10, 50 and 100, each for 50 generations. Compare them for proportion of the populations fixed at generation 50 ( $p = 0$  or 1) and average heterozygosities at generation 50.
2. Using initial frequencies of  $p = 0.9$ ,  $q = 0.1$ , run 50 replicate simulations with population sizes of 10, 50 and 100 each for 50 generations. Compare these results with those from above for proportion of the populations fixed ( $p = 0$  or 1) at generation 50 and average heterozygosities at generation 50.

## Chapter 12 Inbreeding

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Inbreeding is the production of offspring from mating of individuals related by ancestry. It is measured as the probability that two alleles at a locus are identical by descent ( $F$ ). Inbreeding increases homozygosity and exposes rare deleterious alleles

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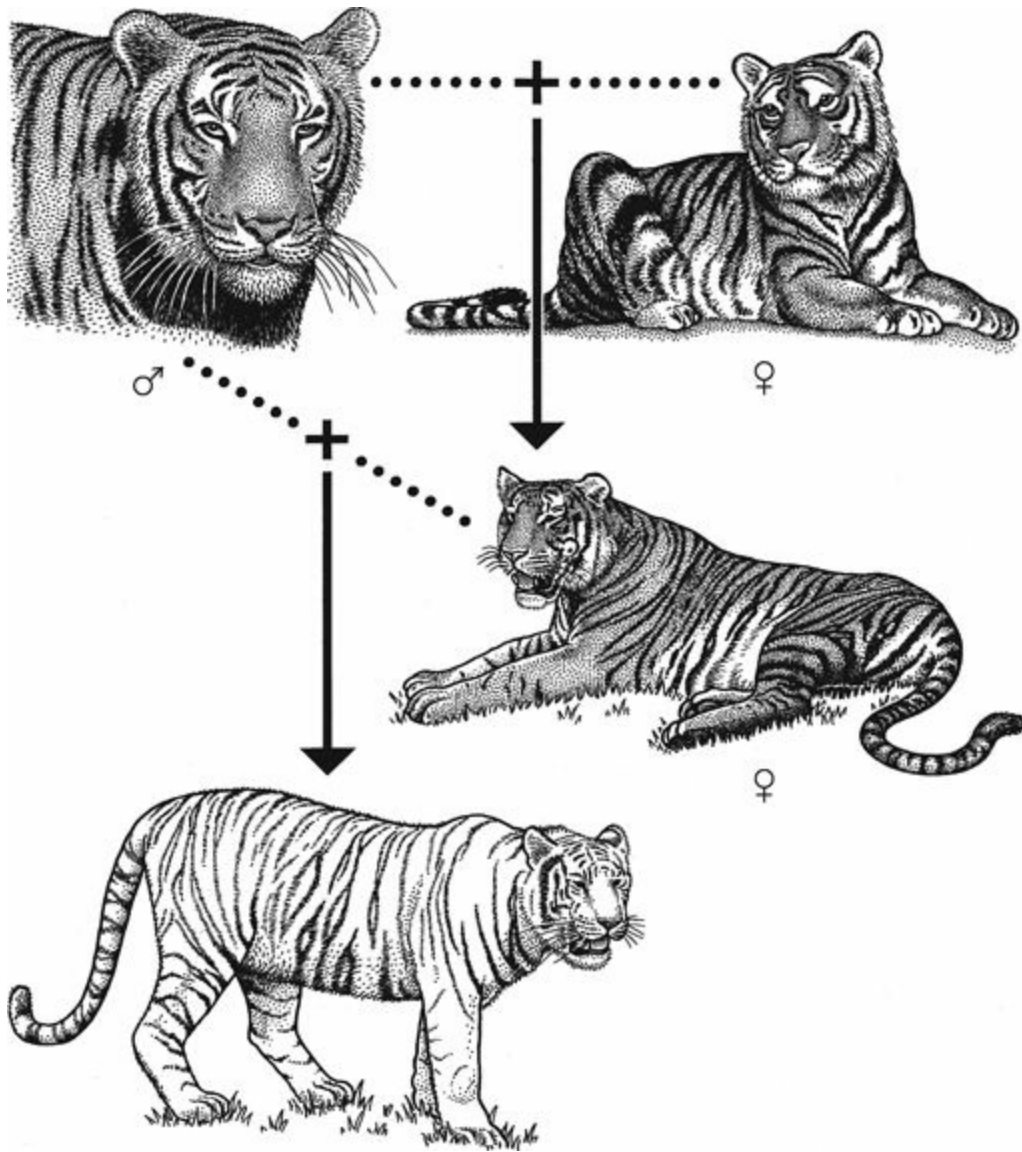
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### **Terms**

Allozygous, autozygous, backcross, base population, common ancestor, full-sib mating, identity by descent, inbreeding coefficient, inbreeding depression, panmictic, pedigree, purging

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Father–daughter mating resulting in a white tiger (India)

**What is inbreeding?**

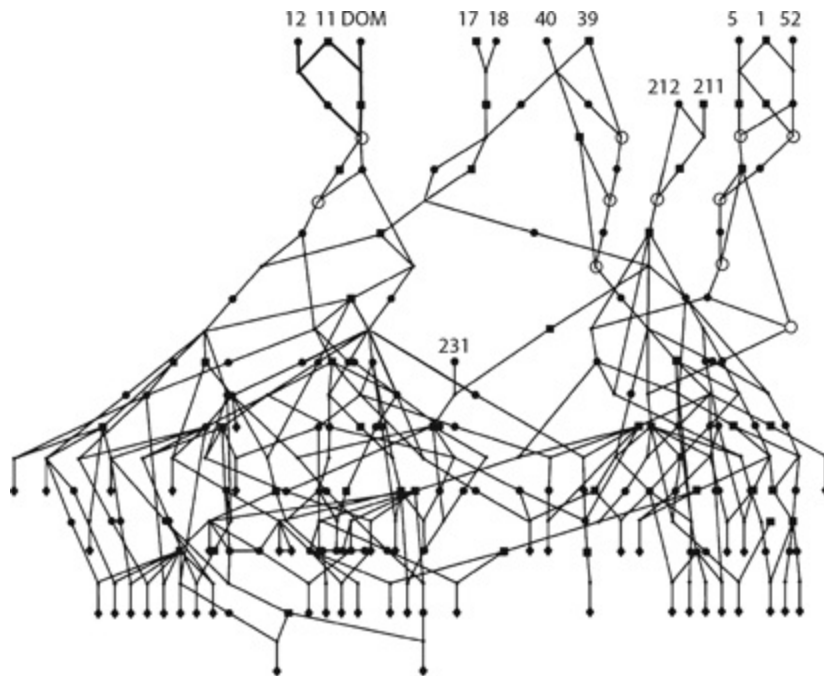
Inbreeding is the production of offspring from mating of individuals related by ancestry

An individual is inbred when its parents share one or more common ancestors (i.e. are related). For example, a father–daughter mating in tigers is illustrated on the chapter frontispiece. Inbred matings include self-fertilization, matings of brother with sister, father with daughter, mother with son, cousins, etc.

Inbreeding is unavoidable in small populations as all individuals become related by descent over time. For example, [Box 12.1](#) shows the early generations of the pedigree for the endangered Przewalski's horse population. As this population is presumed to derive from only 13 individuals, complete avoidance of inbreeding is impossible.

### **Box 12.1 Inbreeding in the endangered Przewalski's horse**

The Przewalski's horse (Mongolian wild horse) became extinct in the wild and existed only in captivity for many years, before being reintroduced into its natural range in Mongolia (Ehrlich & Ehrlich [1981](#)). The population was presumed to derive from only 12 individuals plus one domestic mare (but see below). In the pedigree below (after Thomas [1995](#)), the 13 founders (individuals without connections to ancestors) are numbered; most are near the top of the figure, except for 231, in the middle. DOM is the domestic mare. Circles represent females, squares males and diamonds individuals (or several sibs) which have not yet reproduced.

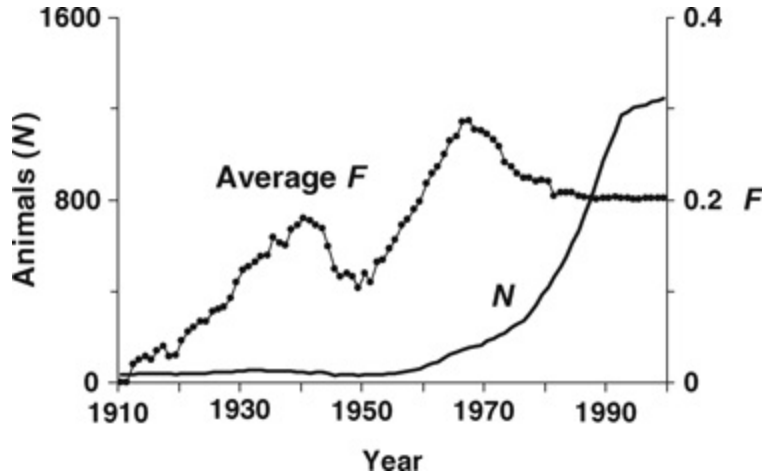


There are many inbred individuals in this pedigree. For example, in the top left-hand side, male 11 produced a daughter with female 12 and a son with DOM; these half-siblings mated to produce an inbred son and an inbred daughter (shown in bold lines on the figure). Analyses indicated that inbreeding was associated with deleterious changes in offspring per mare and longevity in the population (Frankel & Soulé [1981](#)). Genetic management initially minimized the genetic contribution of the domestic mare, but since 1970 has also focused on minimizing inbreeding.

The increases in numbers and average inbreeding are shown below



(after Volf [1999](#)).



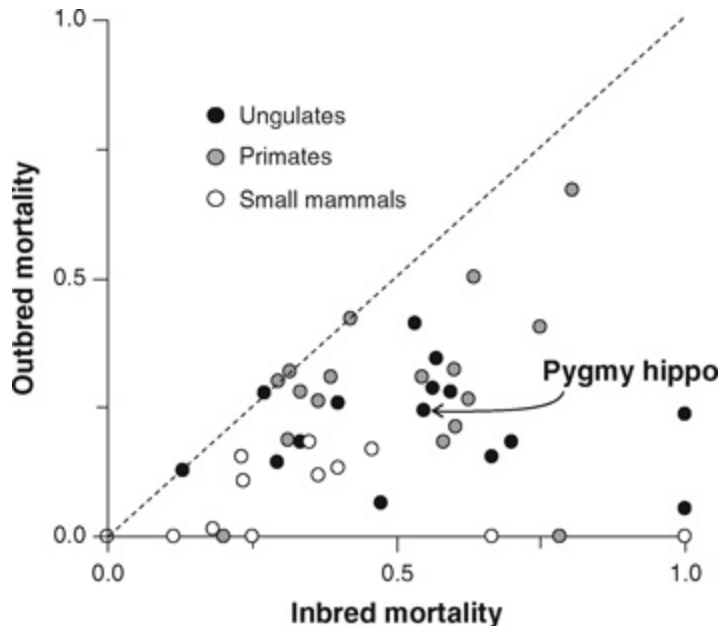
Recent molecular genetic analyses indicate that there are errors in this pedigree and that more than one domestic horse has contributed (Bowling *et al.* [2003](#)).

## Conservation concerns with inbreeding

Inbreeding results in a decline in reproductive fitness (inbreeding depression)

Inbreeding is of profound importance in conservation biology as it reduces reproductive fitness. This is observed in essentially all well-studied populations of outbreeding animals and plants, and increases extinction risk ([Chapter 2](#)). For example, in a study of 44 captive mammal populations, inbred individuals experienced higher juvenile mortality than outbred

individuals in 41 cases (Fig. 12.1). In the pygmy hippopotamus, inbred offspring had 55% juvenile mortality, while outbred offspring had 25% mortality. On average, brother–sister (full-sib) mating resulted in a 33% reduction in juvenile survival.



**Fig. 12.1** Inbreeding depression for juvenile survival in 44 captive mammal populations (Ralls & Ballou 1983). Juvenile mortality in outbred individuals is plotted against that in inbred individuals from the same populations. The line represents equal survival of inbred and outbred individuals. *In most populations inbreeding is deleterious.*

In this chapter we define methods for measuring inbreeding and describe the genetic impacts of inbreeding on genotype frequencies. Further details of inbreeding depression and its causes are deferred until the next chapter.

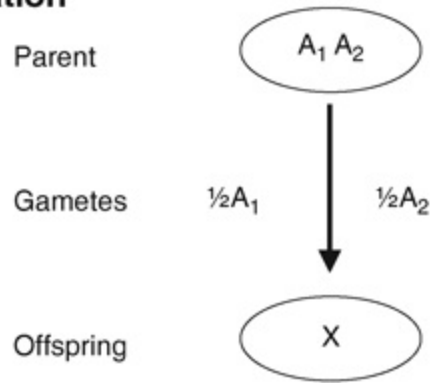
## Measuring inbreeding: inbreeding coefficient ( $F$ )

The inbreeding coefficient of an individual ( $F$ ) is the probability that it carries alleles at a locus that are identical by descent

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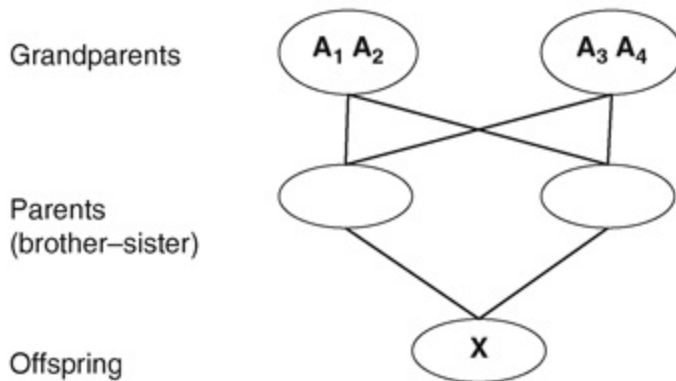
The primary consequence of matings between relatives is that offspring have an increased probability of inheriting alleles that are recent copies of the same DNA sequence (**identical by descent**, or **autozygous**). For example, in [Fig. 12.2](#)  $A_1A_1$  or  $A_2A_2$  offspring resulting from self-fertilization have inherited two alleles which are identical copies of  $A_1$  or  $A_2$  alleles in their parent. The two identical copies of an allele do not need to come from an individual in the previous generation, but may come from a common ancestor in a more remote generation. In [Fig. 12.2](#) an offspring resulting from a brother–sister mating may inherit two copies of allele  $A_1$ ,  $A_2$ ,  $A_3$  or  $A_4$  from its grandparent. The grandparents are said to be **common ancestors**, meaning that they are ancestors of both the mother and the father of the individual.

### Self-fertilization



$$F_X = \Pr(X = A_1 A_1) + \Pr(X = A_2 A_2) \\ = 1/4 + 1/4 = 1/2$$

### Full-sib mating



$$F_X = \Pr(X = A_1 A_1 \text{ or } A_2 A_2 \text{ or } A_3 A_3 \text{ or } A_4 A_4) \\ = 1/16 + 1/16 + 1/16 + 1/16 = 1/4$$

**Fig. 12.2** Inbreeding coefficients for individuals resulting from self-fertilization and full-sib mating.

The probability of both alleles at a locus uniting in an individual are identical by descent is termed the **inbreeding coefficient** ( $F$ ). As  $F$  is a probability, it ranges from 0 in outbreds to 1 in completely inbred individuals.

Identity by descent is related to, but distinct from homozygosity. Some

homozygotes carry two alleles identical by descent (autozygous), while others do not (**allozygous**). For example, in Fig. 3.2 an individual carrying Adh copies 7 and 8 would be homozygous for Adh-F, but these alleles differ in DNA sequence at nine bases, and are not recent copies of the same allele (allozygous). Conversely, an individual carrying two copies of Adh 7 that were inherited from a recent common ancestor of both its parents would be identical by descent (autozygous).

The inbreeding coefficient of an individual resulting from self-fertilization (selfing) is  $\frac{1}{2}$  and that for an individual resulting from brother–sister (full-sib) mating is  $\frac{1}{4}$  (Fig. 12.2). To calculate inbreeding coefficients from first principles, each non-inbred ancestor is labelled as having unique alleles ( $A_1A_2$ ,  $A_3A_4$ , etc.) (Fig. 12.2). The probability that an individual inherits two alleles identical by descent ( $A_1A_1$ , or  $A_2A_2$ , etc.) is computed from the paths of inheritance, assuming normal Mendelian segregation. In a plant that self-fertilizes, the inbreeding coefficient is the probability that offspring X inherits either two  $A_1$  alleles, or two  $A_2$  alleles. Individual X has a  $\frac{1}{2}$  chance of inheriting  $A_1$  in the ovule and a  $\frac{1}{2}$  chance of inheriting it through the pollen, so that the probability that X inherits two identical  $A_1$  alleles is  $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$ . The chance that X inherits two  $A_2$  alleles is also  $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$ . The inbreeding coefficient is then the probability of inheriting either  $A_1A_1$  or  $A_2A_2 = \frac{1}{4} + \frac{1}{4} = 0.5$ . Similarly, an offspring from a brother–sister (full-sib) mating has  $F = 0.25$ .

## Base population

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All inbreeding is measured relative to a base population, but a range of different bases are used

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It is important to understand what **base population** is being used as this affects how  $F$  is calculated, its meaning and its implications on conservation. The source can be the previous generation, a set of parents 10 generations removed, a set of individuals from the mainland founding an island population for the first time or a population 1000 generations ago. The level of inbreeding measured by  $F$  is then the accumulation of inbreeding relative to this base population. For example, an  $F$  can be calculated from a multi-generational pedigree (see below) and it measures the accumulated inbreeding of individuals since the founders of that population. An  $F$  can also be calculated by comparing the observed heterozygosity to expected heterozygosity (see below) and this method uses the previous generation as the base population. The first measures accumulated inbreeding. The latter simply measures deviations from random mating. In the following sections, we will discuss the concept and measurement of  $F$  relative to different base populations, and their implications for conservation genetics.

## Genetic consequences of inbreeding

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Inbreeding increases levels of homozygosity and exposes deleterious recessive alleles

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Naturally outbreeding populations contain deleterious partially recessive alleles at low frequencies in mutation–selection balance ([Chapter 7](#)). Since inbreeding increases homozygosity ([Fig. 4.2](#)) it exposes more deleterious alleles as homozygotes and reduces fitness. This is the primary cause of inbreeding depression. Quantitative expressions for the magnitude of these effects are derived below and in [Chapter 13](#).

We begin with a random mating population containing two alleles, + and  $m$ , at a locus with frequencies of  $p$  and  $q$ . Table 12.1(a) gives the Hardy–Weinberg equilibrium genotype frequencies expected under random mating, while (b) shows the proportions of fully inbred populations that are expected to be homozygous for either + or  $m$ .

**Table 12.1 Genotype frequencies under random mating compared to those in populations with inbreeding coefficients ( $F$ ) of 1 and  $F$**

Populations	$F$	Genotypes		
		+/+	+/ $m$	$m/m$
(a) Random mating ( $N = \infty$ )	0	$p^2$	$2pq$	$q^2$
(b) Fully inbred	1.0	$p$	0	$q$
(c) Partially inbred	$F$	$p^2 (1 - F) + Fp$ $p^2 + Fpq$	$2pq (1 - F) + F \times 0$ $2pq (1 - F)$	$q^2 (1 - F) + Fq$ $q^2 + Fpq$

A partially inbred population (c) consists of genotypes generated in two ways:

- (i) those due to random union of gametes: proportion  $1 - F$
- (ii) those due to union of gametes identical by descent: proportion  $F$ .

The overall genotype frequencies are the sum of these two components. Thus, the frequency of heterozygotes in the next generation is  $2pq (1 - F)$ . The frequency of  $m/m$  homozygotes is  $q^2 (1 - F) + Fq = q^2 + Fpq$  upon rearrangement. Similarly, the frequency of +/+ homozygotes is  $p^2 + Fpq$ . Thus, inbreeding decreases heterozygosity and increases homozygosity.

Inbreeding reduces the frequency of heterozygotes in proportion to the inbreeding coefficient and increases the frequency of each homozygous

type by half of this amount

The ratio of heterozygosity in an inbred population ( $H_{\text{inbred}}$ ) relative to that in a random breeding population ( $H_e$ ) is:

$$\frac{H_{\text{inbred}}}{H_e} = \frac{2pq(1-F)}{2pq} = 1 - F \quad (12.1)$$

Thus, reduction in heterozygosity due to inbreeding over this generation is directly related to the inbreeding coefficient  $F$ . As expected, we observe a deficiency of heterozygotes in populations where there is self-fertilization, as illustrated in [Table 12.2](#).

Inbreeding coefficients can be calculated by comparing the observed to the expected heterozygosity. This measures deviation from random mating but not long-term accumulation of inbreeding

**Table 12.2** *Deficiency of heterozygotes at the locus controlling black versus grey lemma colour in wild oats, a species that often self-fertilizes (Hedrick 2005a after Jain & Marshall). Observed genotype frequencies and those expected with random mating (Hardy–Weinberg equilibrium) are shown*



	Genotype			Allele frequency	
	BB	Bb	bb	$p(B)$	$q(b)$
Observed	0.548	0.071	0.381	0.5835	0.4165
Hardy–Weinberg expectation	0.340	0.486	0.173		

By rearranging [Equation 12.1](#) above, we obtain:

$$F = 1 - \frac{H_o}{H_e} \quad (12.2)$$

From the data in [Table 12.2](#), we estimate from [Equation 12.2](#) that the population had an inbreeding coefficient of  $1 - (0.071/0.486) = 0.85$ .

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Inbreeding *per se* does not change allele frequencies

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The frequencies of the + and m alleles in [Table 12.1](#) remain at  $p$  and  $q$ , respectively (Example 12.1). When inbreeding is due to small population size, allele frequencies in individual populations will change due to genetic drift, but the average frequency over a large number of replicate populations is expected to be unchanged.

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### Example 12.1 Allele frequencies under inbreeding

The frequency of the + allele in the inbred population,  $p_1$ , is obtained by

allele counting, as follows:

$$\begin{aligned}
 p_1 &= \frac{[2 \times \text{freq } (+/+) + \text{freq } (+/m)]}{2} \\
 &= \frac{[2 \times (p^2 + Fpq) + 2pq(1 - F)]}{2} = p^2 + Fpq + pq - Fpq \\
 &= p^2 + pq = p(q + p) = p
 \end{aligned}$$

$$\therefore p_1 = p$$

Thus, there is no change in the frequency of the + allele. The frequency of the  $m$  allele is also unchanged at  $q$ .

The relative increase in homozygote frequencies for deleterious alleles depends on the  $F$  and the allele frequency

The ratio of frequencies of recessive homozygotes under inbreeding versus random mating is

$$\text{ratio} = \frac{(q^2 + Fpq)}{q^2} = \frac{1 + Fp}{q} \quad (12.3)$$

where  $q$  is the frequency of the recessive allele. Thus, the ratio increases with the amount of inbreeding and is greater for rare than for common alleles.

For example, the frequency of homozygous  $dw/dw$  in California condors is more than doubled in a population with an inbreeding coefficient of 25% and

almost six-fold higher in a completely inbred population ([Table 12.3](#)).

**Table 12.3 Expected genotype frequencies under inbreeding at the chondrodystrophy locus in California condors. The deleterious recessive allele has a frequency of ~17%, and the normal allele ~83% (Example 4.5). Genotype frequencies are shown for random mating, complete inbreeding and full-sib mating ( $F = 0.25$ ) determined using the formulae in [Table 12.1](#). The ratios of frequencies of  $dw/dw$  in inbred to outbred populations are also given**

	Genotypes			Ratio
	$+/+$	$+/dw$	$dw/dw$	
Random mating	0.6889	0.2822	0.0289	
Partially inbred $F = 0.25$	0.7242	0.2116	0.0642	2.2
Inbred $F = 1$	0.83	0	0.17	5.9

Lethal allele frequencies are usually much lower than 17%. A more typical frequency for a partially recessive lethal allele in mutation–selection balance is  $\sim 5 \times 10^{-4}$  ([Chapter 7](#)). In a population with an inbreeding coefficient of 25% due to full-sib mating, the ratio of the lethal homozygote frequency to that with no inbreeding would be

$$\text{ratio} = 1 + \frac{Fp}{q} = 1 + \frac{[0.25 \times (1 - 5 \times 10^{-4})]}{5 \times 10^{-4}} = 501$$

i.e. the frequency of lethal homozygotes after one generation of full-sib mating would be about 500 times higher than with random mating.

The frequency of lethal homozygotes under random mating ( $q^2$ ) is  $25 \times 10^{-8}$  when  $q = 5 \times 10^{-4}$ , while that following full-sib mating ( $q^2 + Fpq$ ) is  $25 \times 10^{-8} + 1.25 \times 10^{-4}$ . Most of the lethal homozygotes in the inbred population derive from the inbreeding. While the increased frequency of the lethal genotype is very small at any single locus, similar effects occur across all loci in the genome that are segregating for lethal alleles.

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Inbreeding has large cumulative effects across all loci in the genome

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There are ~20 000 protein coding loci in the mammalian genome and at least 5000 can produce lethal mutations in mice (Miklos & Rubin 1996). The cumulative impacts of inbreeding on 5000 loci, indicate that about 47% of all zygotes from full-sib matings will be homozygous for at least one such lethal allele (Box 12.2). In addition, there will be many other less deleterious alleles, whose homozygosity will reduce the reproductive fitness of inbred populations. The value of 47% is greater than the 33% reduction in juvenile survival found for full-sib mating in mammals (Ralls & Ballou 1983). However, many lethals will cause zygotic mortality prior to birth and not be recorded as juvenile mortality.

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### **Box 12.2 Overall frequency of individuals homozygous for lethal alleles in inbred populations**

At least 5000 loci can mutate to produce lethal alleles in mice and, presumably, other mammals. The typical mutation–selection equilibrium frequency for a partially recessive lethal is  $5 \times 10^{-4}$  (Chapter 7).

The probability ( $P$ ) that a zygote is *not* homozygous for a lethal at a single locus under random mating is  $1 - q^2$ . To obtain the probability for the whole genome, we raise this to the power of the number of loci likely to be segregating for lethal alleles (5000 in this case). Thus:

$$P(\text{not a lethal homozygote}) = (1 - q^2)^{5000}$$

and after substituting for  $q$ , we have

$$P(\text{not a lethal homozygote}) = [1 - (5 \times 10^{-4})^2]^{5000} = 0.99875$$

Thus, only about 0.125% of zygotes will be homozygous for a lethal in a large random mating population.

Under full-sib mating, the probability that an individual is not homozygous for a lethal is  $1 - q^2 - Fpq$  at each locus and the probability that it is not homozygous for a lethal anywhere in the genome is:

$$P(\text{not lethal homozygote}) = (1 - q^2 - Fpq)^{5000} \sim (1 - q^2 - Fq)^{5000}$$

(as  $p \sim 1$ ). For progeny of full-sib matings, ( $F = 0.25$ ) this probability is:

$$P(\text{not lethal homozygote}) = [1 - (5 \times 10^{-4})^2 - (0.25 \times 5 \times 10^{-4})]^{5000} = 0.53$$

Consequently, the probability that an individual is homozygous for lethal alleles at one or more loci is  $1 - 0.53 = 0.47$ .

---

Increased homozygosity of deleterious partially recessive alleles provides an obvious mechanism by which inbreeding reduces reproductive fitness in naturally outbreeding species (see [Chapter 13](#)).

We next consider the rise in levels of inbreeding in small closed random mating populations.

## Inbreeding in small random mating populations

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Inbreeding is unavoidable in small closed random mating populations

In small, closed random mating populations all individuals eventually become related by descent and inbreeding is unavoidable, as is evident in the pedigree for Przewalski's horse ([Box 12.1](#)). The number of ancestors rapidly exceeds the historical population size and individuals must have common ancestors. For example, 10 generations ago each of us had 1024 ancestors. Consequently, if the historic population size was less than 1024 times the current population size, then individuals in a closed population must be inbred to some degree.

## Theory of inbreeding in small populations

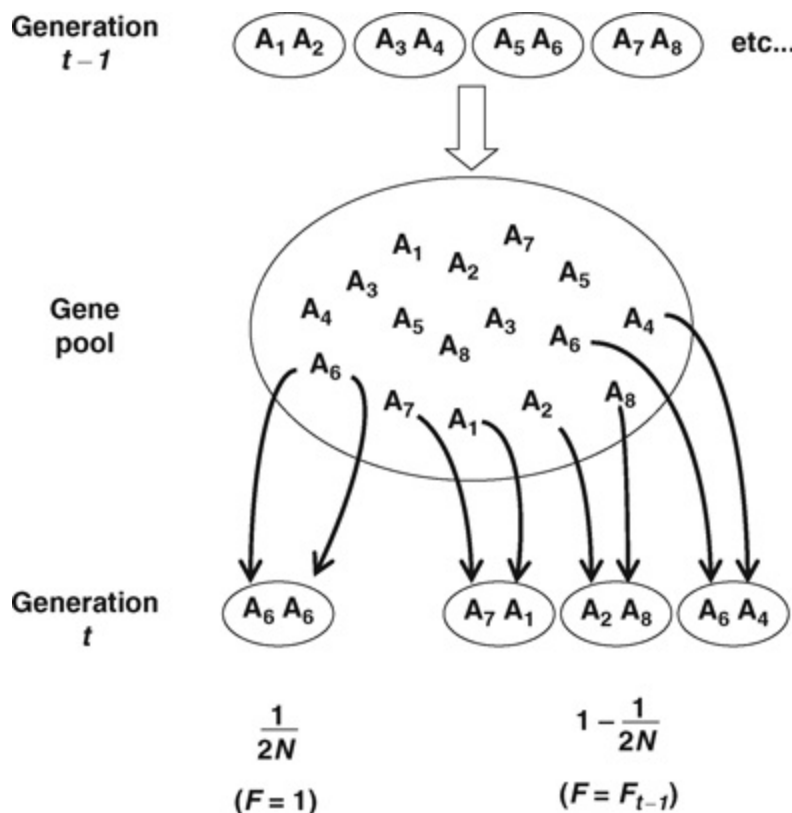
Inbreeding in a random mating population of size  $N_e$  increases at a rate of  $1/(2N_e)$  per generation

We derive expressions for the effects of population size on inbreeding by determining changes over generations in the probability of identity by descent in an idealized random mating population ([Chapter 11](#)). We assume that all founding individuals (generation 0) are non-inbred, unrelated and carry unique alleles. For a hermaphroditic marine species that sheds gametes into the sea, there are  $N$  individuals producing equal numbers of gametes that unite at random, and  $2N$  ancestral alleles  $A_1, A_2, A_3, \dots, A_{2N}$  in the gene

pool. Each individual in generation 1 is formed by sampling, with replacement, two alleles at random from this pool. If the first allele sampled is  $A_6$ , the probability that the second is also  $A_6$  (identical by descent) is  $1/(2N)$ . For all individuals, the probability that they have two alleles identical by descent is  $1/(2N)$ . Consequently, the inbreeding coefficient in the first generation is  $1/(2N)$ .

In following generations, there are two ways that identical alleles can be sampled to create a zygote (Fig. 12.3):

- from the sampling of two copies of the same allele (as above) with probability  $1/(2N)$  (new inbreeding)
- from sampling two alleles that are identical from previous inbreeding.



**Fig. 12.3** Inbreeding due to small population size. Different genotypes in generations  $t-1$  and  $t$  are shown in the small ellipses above and below, respectively. Taking a random sample of  $2N$  gametes from the generation  $t-1$  gene pool involves a probability of  $1/(2N)$  that two identical alleles are sampled and a probability of  $1 - 1/(2N)$  that two distinct alleles are sampled.

The probability of sampling two different alleles is  $1 - 1/(2N)$ , and a proportion  $F$  of these alleles is identical by descent due to the previous inbreeding. Thus, the contributions due to previous inbreeding is  $F_{t-1} \times [1 - 1/(2N)]$ .

Taken together the probability of creating a zygote in generation  $t$  with both alleles identical by descent ( $F_t$ ) is the sum of these:

$$F_t = \frac{1}{2N} + \left(1 - \frac{1}{2N}\right)F_{t-1} \quad (12.4)$$

where  $F_{t-1}$  is the inbreeding coefficient in the previous generation,  $t-1$ . Even if there is no new inbreeding, as may occur if population size increases dramatically, the population does not lose its previous inbreeding.

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Inbreeding is more rapid in small than in large populations

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From above, the increment in inbreeding per generation (the rate of inbreeding), is:

$$\Delta F = \frac{1}{2N} \quad (12.5)$$

Consequently, the rate of inbreeding is inversely proportional to population size. Recall from [Equation 8.2](#) that loss of heterozygosity in one generation is also  $1/(2N)$ . Thus, the increment in inbreeding equals the loss of heterozygosity per generation, illustrating the intimate relationship between



inbreeding and loss of genetic diversity in random mating species.

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Inbreeding accumulates over generations in closed random mating populations

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So far, we have expressed the inbreeding coefficient as a function of that in the previous generation. However, we often wish to predict the accumulation of inbreeding. If the population size is constant over generations, we can obtain the required expression for  $F_t$  by rearranging [Equation 12.4](#):

$$1 - F_t = 1 - \frac{1}{2N} + \left(1 - \frac{1}{2N}\right)F_{t-1} = \left(1 - \frac{1}{2N}\right)(1 - F_{t-1})$$

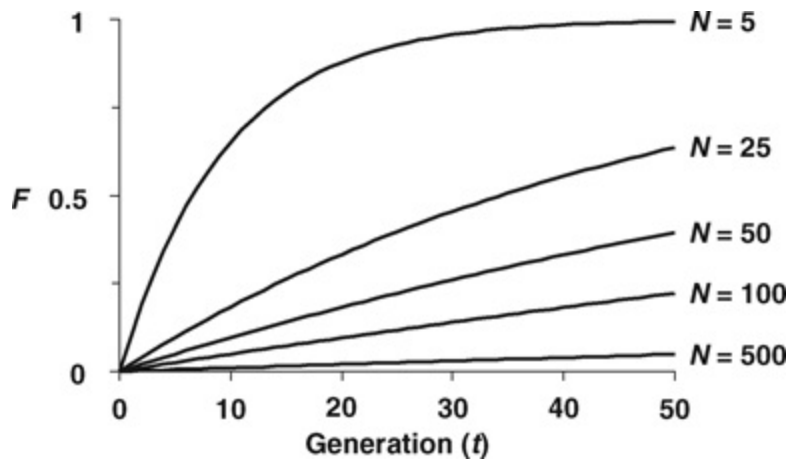
and

$$1 - F_t = \left(1 - \frac{1}{2N}\right)^t (1 - F_0)$$

When the base population is not inbred ( $F_0 = 0$ ), the accumulated inbreeding coefficient at any subsequent generation  $t$ , is:

$$F_t = 1 - \left(1 - \frac{1}{2N}\right)^t \tag{12.6}$$

Thus, inbreeding accumulates with time in all closed finite populations ([Fig. 12.4](#)). This equation applies strictly to an idealized population where selfing occurs at a rate specified by the random union of gametes. With separate sexes and no selfing, there is no inbreeding until generation 2 and the rate of accumulation is a little less (see Crow & Kimura [1970](#)).



**Fig. 12.4** Increase in inbreeding coefficient  $F$  with generations in finite populations of different sizes ( $N$ ). *Inbreeding increases more rapidly in smaller than in larger populations.*

As an example, a small closed population with only four individuals per generation reaches an average inbreeding coefficient of 74% by generation 10 (Example 12.2). This is approximately equivalent to the level of inbreeding due to two generations of selfing or six generations of full-sib mating – but it was achieved in a random mating population. Since captive populations of endangered animals within individual zoos are often of this size, individuals or semen have to be moved between institutions to avoid rapid inbreeding ([Chapter 19](#)).

### **Example 12.2 Accumulation of inbreeding in a small closed captive population**

Many captive populations of threatened animal species in individual zoos are small. If a zoo started a breeding program with four unrelated *Partula* snails, and kept the breeding population at four parents per generation over many generations without adding immigrants, the inbreeding coefficient would increase as follows:

Generation 0  $F = 0$

Generation 1  $F = 1 - \left(1 - \frac{1}{2N}\right)^1 = 1 - \left(1 - \frac{1}{8}\right) = 0.125$

Generation 2  $F = 1 - \left(1 - \frac{1}{2N}\right)^2 = 1 - \left(1 - \frac{1}{8}\right)^2 = 0.23$

Generation 3  $F = 1 - \left(1 - \frac{1}{2N}\right)^3 = 1 - \left(1 - \frac{1}{8}\right)^3 = 0.33$

Generation 10  $F = 1 - \left(1 - \frac{1}{2N}\right)^{10} = 1 - \left(1 - \frac{1}{8}\right)^{10} = 0.74$

Thus, the inbreeding coefficient increases rapidly and reaches 74% by generation 10. The above treatment is only approximate as most threatened animals in zoos do not self, so there is no inbreeding until generation 2. However, this makes little difference after several generations.

For populations that do not have idealized structures,  $N_e$  is used in place of  $N$  in prediction equations

If the population does not have the structure of an idealized population (and few, if any, will) the increase in  $F$  in any population of constant size can be obtained by substituting  $N_e$  for  $N$  in the earlier equations.

For the case of populations that fluctuate in size among generations (as most do), the expression for the inbreeding coefficient at generation  $t$  is:

$$F_t = 1 - \prod_{i=1}^t \left(1 - \frac{1}{2N_{ei}}\right)$$

(12.7)

where  $N_{ei}$  is the effective size in the  $i$ th generation. Example 12.3 illustrates the use of this equation to estimate the minimum inbreeding level in the northern elephant seal following a bottleneck of 20–30 individuals. Alternatively, the harmonic mean  $N_e$  could be used in [Equation 12.6](#) to give the same result.

---

### Example 12.3 Inbreeding in a fluctuating population

The northern elephant seal declined to 20–30 individuals and has since recovered to over 175 000. If we consider, for simplicity, that its effective numbers in three successive generations were 175 000, 20 and 175 000 then its inbreeding coefficient from [Equation 12.7](#) would be

$$\begin{aligned} F_t &= 1 - \prod_{i=1}^t \left( 1 - \frac{1}{2N_{ei}} \right) \\ &= 1 - \left[ \left( 1 - \frac{1}{2 \times 175000} \right) \times \left( 1 - \frac{1}{2 \times 20} \right) \times \left( 1 - \frac{1}{2 \times 175000} \right) \right] \\ &= 1 - 0.975 = 0.025 \end{aligned}$$

Thus, the northern elephant seal has an inbreeding coefficient of at least 2.5% as a result of its previous bottleneck, even though its current size is over 175 000 individuals. Essentially all of its inbreeding is due to the generation of minimum size. The actual inbreeding level will be much greater than this, as it has a polygamous mating system and existed at small population sizes for much more than a single generation.

---

## Indirect estimates of population inbreeding coefficients

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An estimate of accumulated inbreeding can be obtained from the ratio of expected heterozygosities over time

In most populations, levels of inbreeding are unknown. However, an estimate of the accumulated inbreeding coefficient in a population can be obtained from the loss of genetic diversity over time. From [Equations 11.1](#) and [12.6](#), the ratio of expected heterozygosity of a base population when  $t = 0$  ( $H_0$ ) to the expected heterozygosity at a later generation  $t$ , ( $H_t$ ) is

$$\frac{H_t}{H_0} = \left(1 - \frac{1}{2N_e}\right)^t = 1 - F \quad (12.8)$$

The level of inbreeding that has accumulated over time ( $F$ ) can be measured as

$$F = 1 - \frac{H_t}{H_0} \quad (12.9)$$

[Box 12.3](#) illustrates estimation of the effective inbreeding coefficient in the endangered Isle Royale population of gray wolves in North America. The estimate of 55% indicates that this endangered island population is highly inbred, as its history would lead us to suspect.

**Box 12.3 Inbreeding due to low founder numbers and small population sizes in the endangered population of Isle Royale gray wolves (after Wayne et al. [1991](#))**

Gray wolves became established (presumably by a single pair) on Isle Royale in Lake Superior in about 1949, during an extreme winter when

the lake froze. Moose, their main prey, had previously become established on the island. The wolf population rose to 50 in 1980, but subsequently declined to 14 in 1990. This decline could have been due to reduced availability of prey, disease, deleterious effects of inbreeding, or a combination of these factors.



The island population is inbred due to low founder numbers and subsequent small population sizes (two to three breeding pairs). Allozyme heterozygosity, based on 25 loci, was 3.9% for Isle Royale, compared to 8.7% in wolves from nearby mainland populations (used as  $H_0$ ). Using [Equation 12.9](#), the effective inbreeding coefficient is

$$F_e = 1 - \frac{H_{\text{island}}}{H_{\text{mainland}}} = 1 - \frac{0.039}{0.087} = 0.55$$

Consequently, this endangered island population is highly inbred. As gray wolves suffer reductions in reproductive fitness due to inbreeding (Laikre & Ryman [1991](#); Liberg *et al.* [2005](#)), this may be a factor in their decline.

---

Many polymorphic loci should be used to estimate effective inbreeding coefficients, as there is wide variation in homozygosity among loci due to the chance effects involved in Mendelian segregation.

Deviations of genotype frequencies from Hardy–Weinberg equilibrium for

allozyme or DNA markers are widely used to determine selfing rates in plants ([Chapter 21](#)).

## Pedigrees

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Simple methods exist for determining inbreeding from pedigrees

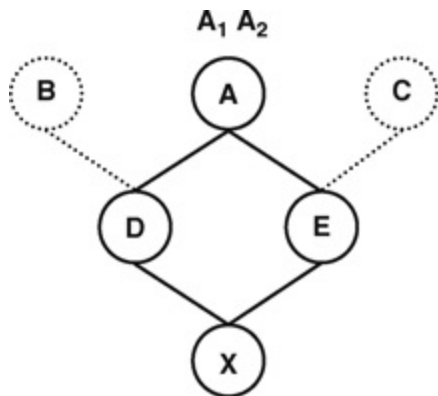
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Where available, pedigrees can be used to determine the inbreeding coefficients of individuals. This allows us to evaluate the effects of inbreeding on survival or reproduction rates, etc. using data from individuals with different levels of inbreeding. Computation of  $F$  from first principles becomes impractical when dealing with complex pedigrees such as that for Przewalski's horse ([Box 12.1](#)). Consequently, simpler alternative methods have been devised for use with complex pedigrees.

We begin by considering an example where we can determine the inbreeding coefficient from first principles, and then illustrate how the method can be simplified for more complex pedigrees. [Figure 12.5](#) illustrates a pedigree where there is mating between half-sibs. The parents of individual X are only related through their common parent A (genotype  $A_1A_2$ ), so we only have to consider the transmission of alleles from A through D and E to X. The inbreeding coefficient of individual X ( $F_X$ ) is determined by computing the probability that individual X is either  $A_1A_1$  or  $A_2A_2$ , i.e. that it receives two alleles identical by descent. The probability that  $A_1$  is transmitted from grandparent A to parent D is  $\frac{1}{2}$ , and that it is then transmitted from D to X is a further  $\frac{1}{2}$ . Similarly, the probability that  $A_1$  is passed from A to parent E is  $\frac{1}{2}$ , and from E to X another  $\frac{1}{2}$ . Thus, the

probability that X is  $A_1A_1$  is the product of the probabilities for four paths:

$$\Pr(X = A_1A_1) = (\frac{1}{2})^4 = \frac{1}{16}$$



**Fig. 12.5** Pedigree with mating between half-sibs.  $\Pr(A_1A_1) = 1/16$   
 $\Pr(A_2A_2) = 1/16$   $\Pr(A_1A_2) = 1/8$ .

Similarly, the probability that X is  $A_2A_2$  is  $(\frac{1}{2})^4 = 1/16$ , and:

$$F_X = \Pr(X = A_1A_1 \text{ or } A_2A_2) = \frac{1}{16} + \frac{1}{16} = \frac{1}{8}$$

The probability of X being homozygous represents the new inbreeding arising from A as a common ancestor.

However, if A is already inbred (i.e.  $A_1$  and  $A_2$  have, in reality, a probability  $F$  of being identical by descent), an additional amount of homozygosity occurs. Individuals inheriting  $A_1$  and  $A_2$  may be inheriting identical alleles. The probability of an offspring inheriting  $A_1A_2$  is:

$$\Pr(X = A_1A_2) = (\frac{1}{2})^4 + (\frac{1}{2})^4 = (\frac{1}{2})^3$$

The probability that  $A_1$  is identical by descent with  $A_2$  is A's inbreeding coefficient  $F_A$ . Thus, the probability that X is identically homozygous through previous inbreeding is:

$$\Pr(X \text{ is identically homozygous through past inbreeding}) = (\frac{1}{2})^2 F_A$$



The overall inbreeding coefficient of X due to both new and previous inbreeding is:

$$F_X = (\frac{1}{2})^3 + (\frac{1}{2})^3 F_A = (\frac{1}{2})^3 (1 + F_A)$$

Thus, if a common ancestor is already inbred, inbreeding is increased further in later generations.

The inbreeding coefficient for an individual can be calculated from the number of individuals in each path connecting one parent to the other through each common ancestor, and the  $F$  of each common ancestor

The inbreeding coefficient can be obtained more simply by counting the individuals in the path from mother to father through the common ancestor (including both parents), and raising  $\frac{1}{2}$  to this power. The  $\frac{1}{2}$  reflects the probability that an allele is transmitted from one generation to the next, and the power reflects the number of such parent–offspring steps in the pedigree. For example, in [Fig. 12.5](#) there are three individuals connecting parents of individual X through their common ancestor A, i.e. through D, A and E. The inbreeding coefficient of X is  $(\frac{1}{2})^3$ , if A is not inbred (as obtained above).

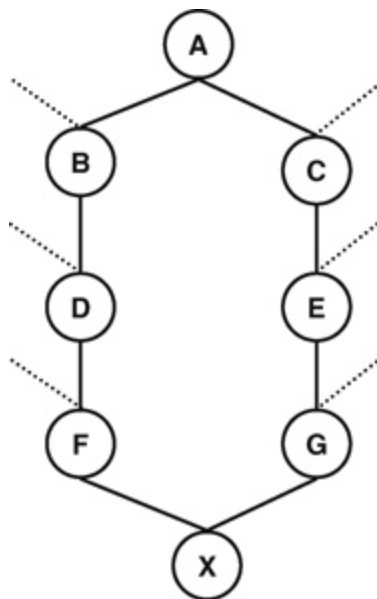
In more complex pedigrees, the parents of an individual may be related through more than one common ancestor, or from the same ancestor through different paths. Each common ancestor, and each path, contributes an additional probability of the progeny having identity by descent. The inbreeding coefficient is the sum of the probabilities, as follows:

$$F = \Sigma (\frac{1}{2})^n (1 + F_{ca})$$

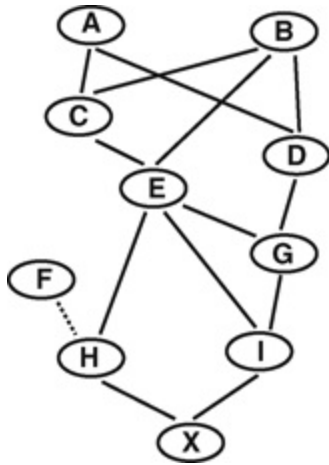
(12.10)

where  $n$  is the number of individuals in the path from one parent to a common ancestor and back to the other parent, and  $F_{ca}$  is the inbreeding coefficient of that particular common ancestor. These contributions to inbreeding are summed for each different path linking both parents to each common ancestor.

We apply this method to the simple pedigree in Fig. 12.6 and then to a more complex case in Fig. 12.7. In Fig. 12.6, the individuals to include from one parent to the other via the common ancestor (A) are F, D, B, A, C, E and G, making  $n = 7$  steps. Thus  $F_X$  in Fig. 12.6 is  $(\frac{1}{2})^7 (1 + F_A) = 1/128$ , if individual A is not inbred.



**Fig. 12.6** Pedigree with a more remote common ancestor. The dotted lines represent paths to other ancestors that are not on the path to the common ancestor A.



**Fig. 12.7** Complex pedigree for male Dorcas gazelle 102796 (X) at the National Zoological Park, Washington, DC.

Calculation of the inbreeding coefficient for the Dorcas gazelle shown in [Fig. 12.7](#) involves summing six different paths, two of them involving inbred common ancestors (Example 12.4). The inbreeding coefficient is 0.266, with the paths to the most recent common ancestor (E), making the greatest contribution. Distant common ancestors contribute little to the inbreeding coefficient.

#### **Example 12.4 Determining the inbreeding coefficient for Dorcas gazelle X in the pedigree shown in [Fig. 12.7](#)**

Individuals A, B and E are common ancestors creating relationships between H and I, the parents of individual X. The paths of relationship, the number of individuals in paths joining parents through common ancestors ( $n$ ), the inbreeding coefficients of common ancestors, and the contribution of each of the paths to the inbreeding coefficient  $F_X$  (rounded to four decimal places), are shown below. A, B and F are all assumed to be unrelated and non-inbred. Individual E is the result of a parent (B) – offspring (C) mating and has an inbreeding coefficient of  $(\frac{1}{2})^2 = 0.25$ . The overall inbreeding coefficient of X is 0.2656.

Paths of relationship (common ancestors are in bold)	<i>n</i>	<i>F</i> of common ancestor	Contribution to $F_x$
HEC <b>A</b> DGI	7	0	$(\frac{1}{2})^7 = 0.0078$
HEC <b>B</b> DGI	7	0	$(\frac{1}{2})^7 = 0.0078$
HE <b>B</b> DGI	6	0	$(\frac{1}{2})^6 = 0.0156$
HE <b>G</b> I	4	$\frac{1}{4}$	$(\frac{1}{2})^4 \times \frac{5}{4} = 0.0781$
HE <b>I</b>	3	$\frac{1}{4}$	$(\frac{1}{2})^3 \times \frac{5}{4} = 0.1563$
			$F_x = 0.2656$



Dorcas gazelle

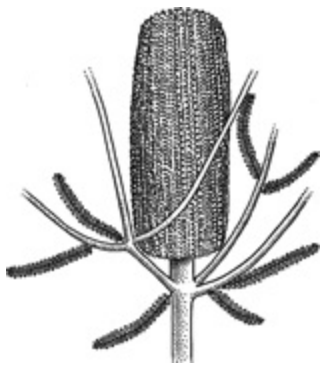
For complex pedigrees, inbreeding coefficients are typically calculated using computer programs (Pollak *et al.* [2009](#)).

## Regular systems of inbreeding

Some species routinely reproduce by selfing

Self-fertilization is common in plants; 20% are highly selfing and another

33% are intermediate between selfing and outcrossing (Vogler & Kalisz 2001; Barrett 2002). Further, self-fertilization occurs in some hermaphroditic reef corals and in some snails and slugs, and a few animals sib mate regularly (Selander 1983; Carlon 1999). For example, endangered Brown's banksia from Western Australia exhibits about 30% self-fertilization (Sampson *et al.* 1994), while a brain coral on the Great Barrier Reef shows about 50% selfing (Stoddart *et al.* 1988). Consequently, we need to consider the outcomes of repeated deliberate inbreeding over generations.



Brown's banksia



Brain coral

Figure 12.8 illustrates the increase in inbreeding over generations due to selfing, full-sib mating and backcrossing to a parent. The recurrence relationships for these cases are given in Table 12.4. The use of the recurrence relationship for a selfing plant is shown in Example 12.5 and that for full-sib mating in Example 12.6.

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<b>Example 12.5 Accumulation of inbreeding over</b>
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## generations in a selfing plant species

The rise in inbreeding coefficient for a species with continual selfing is computed using the recurrence relationship from [Table 12.4](#):

$$F_t = \frac{1}{2}(1 + F_{t-1})$$

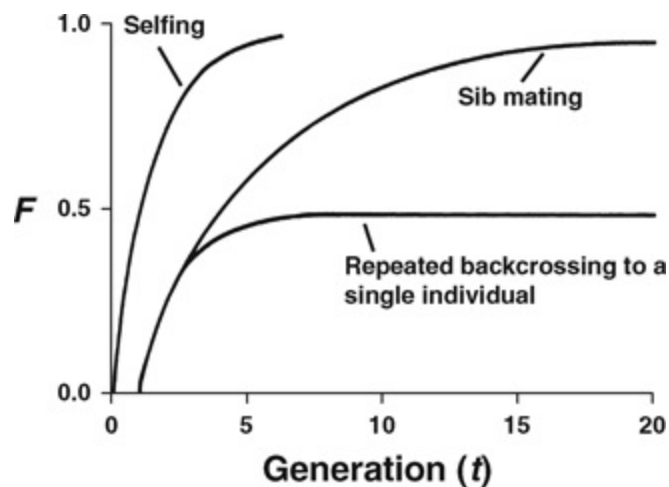
If the initial population is non-inbred,  $F_{t-1} = 0$ , then

$$F_1 = \frac{1}{2}(1 + 0) = 0.5$$

$$F_2 = \frac{1}{2}(1 + 0.5) = 0.75$$

$$F_3 = \frac{1}{2}(1 + 0.75) = 0.875$$

and the population rapidly approaches  $F \sim 1$  (complete inbreeding).



**Fig. 12.8** Increase in inbreeding coefficients arising from repeated generations of selfing, full-sib mating and backcrossing to a single individual.

**Table 12.4** *Recurrence relationships for regular systems of inbreeding*

Form of inbreeding	Recurrence relationship <sup>a</sup>
Self-fertilization	$F_t = \frac{1}{2} (1 + F_{t-1})$
Full-sib mating	$F_t = \frac{1}{4} (1 + 2F_{t-1} + F_{t-2})$
Repeated backcrossing to a single individual, A	$F_t = \frac{1}{4} (1 + F_A + 2F_{t-1})$

<sup>a</sup>  $F$  is the inbreeding coefficient and  $t$  is the generation (derivations given in Falconer & Mackay 1996).

### Example 12.6 Accumulation of inbreeding with repeated full-sib mating

In a few animal species, such as the colonial naked mole rat, full-sib inbreeding is a regular part of the mating system (Reeve *et al.* 1990), while repeated full-sib mating is used to generate inbred populations of laboratory animals for experimental purposes. The rise in  $F$  is calculated using the recurrence relationship from Table 12.4, as follows:

$$F_t = \frac{1}{4}(1 + 2F_{t-1} + F_{t-2})$$

If we assume no prior inbreeding ( $F_{t-1}$  and  $F_{t-2} = 0$ ), then

$$F_1 = \frac{1}{4}(1 + 0 + 0) = 0.25$$

$$F_2 = \frac{1}{4}(1 + 2 \times 0.25 + 0) = 0.375$$

$$F_3 = \frac{1}{4}(1 + 2 \times 0.375 + 0.25) = 0.5$$

Consequently, the inbreeding coefficient with full-sib mating reaches a value of 0.5 after three generations, the same value achieved after one generation of selfing. The inbreeding coefficient rises with continued sib mating until  $F \sim 1$  after about 20 generations.

## Backcrossing

Backcrossing can be used to recover sub-species that have been reduced to a single individual (Fig. 12.9). For example, the one remaining female Norfolk Island boobook owl has been crossed to its nearest related sub-species from New Zealand. The survivor A is mated to B (from a different sub-species), and the offspring in this and subsequent generations backcrossed to A. Backcrossing to the original parent increases the genetic representation of the threatened sub-species in the offspring, but is done at the expense of increased inbreeding. If the boobook owl female had lived long enough to be used for four generations of backcrossing, the progeny would have had an inbreeding coefficient averaging ~50% (Example 12.7).

### Example 12.7 Inbreeding with repeated backcrossing to one individual

The one remaining female Norfolk Island boobook owl has been crossed to males from the related New Zealand sub-species. By backcrossing to the female, wildlife managers could generate a population with a gene pool consisting mostly of Norfolk Island boobook alleles. What effect will this have on the inbreeding coefficients of the progeny?





Let us first assume that the one remaining female owl is not inbred. The hybrid offspring will have an inbreeding coefficient of 0. The progeny of the first-generation backcross of the hybrids to the Norfolk Island female will have an inbreeding coefficient:

$$F_1 = 0.25 \text{ (since } F_{t-1} = 0)$$

$$F_2 = \frac{1}{4}(1 + 2F_1) = \frac{1}{4}(1 + 1/2) = 0.375$$

$$F_3 = \frac{1}{4}(1 + 2F_2) = \frac{1}{4}(1 + 3/4) = 0.4375$$

$$F_4 = \frac{1}{4}(1 + 2F_3) = \frac{1}{4}(1 + 7/8) = 0.46875$$

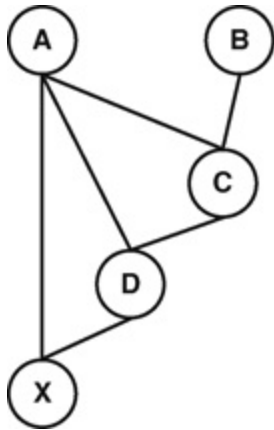
Thus, the regenerated boobook owl population would have an inbreeding coefficient of almost 47% after four generations of backcrossing.

The proportion of the genotype deriving from the Norfolk Island boobook owl is 50% in the first cross and 75%, 87.5%, 93.75% and 96.9% after 1, 2, 3 and 4 generations of backcrossing, respectively.

If the one remaining female owl was herself the offspring of a full-sib mating ( $F_A = \frac{1}{4}$ ), the first-generation hybrid offspring would still have an  $F = 0$ , but the following generations will have higher inbreeding coefficients than above, i.e.

$$F_1 = \frac{1}{4}(1 + \frac{1}{4}) = 0.3125$$

and the inbreeding coefficient in the fourth generation would be almost 59%. In reality, the one remaining owl is likely to be partially inbred.



**Fig. 12.9** Pedigree for repeated backcrossing to the same individual.

## Mutation–selection balance with inbreeding

Selfing species are expected to have lower equilibrium frequencies for deleterious alleles

Inbreeding increases the frequency of homozygotes and increases the opportunity for deleterious recessive alleles to be removed by selection (**purging**). Thus, mutation–selection equilibrium frequencies for inbreeding species are lower than those for outbreeding species ([Table 12.5](#)). This is illustrated for lethal alleles with differing degrees of dominance in [Example 12.8](#). The reduction in frequency of a complete recessive lethal allele is most pronounced and somewhat less for a partial recessive. With very high levels of inbreeding (such as continual selfing), the equilibrium frequency for all cases (irrespective of the level of dominance) is approximately  $u/s$ . However, the situation is rather different for mildly deleterious alleles in small populations where purging has very little impact on their removal under full-sib inbreeding ([Hedrick 1994](#)).

**Table 12.5 Mutation–selection equilibria for deleterious alleles, comparing selfing with random mating.  $u$  is the mutation rate,  $s$  the selection coefficient against deleterious homozygotes,  $hs$  the selection coefficient against heterozygotes, and  $F$  the inbreeding coefficient**

	Inbreeding	Random mating
Complete recessive	$\sim \frac{\sqrt{(F^2 + 4u/s)} - F}{2}$	$\sqrt{\frac{u}{s}}$
Partial recessive	$\sim \frac{u}{[s(h + F)]}$	$\frac{u}{hs}$
Highly inbred (irrespective of dominance)	$\sim \frac{u}{s}$	

Sources: Li (1955); Crow & Kimura (1970).

### Example 12.8 Comparison of mutation–selection equilibria for lethal alleles under inbreeding versus random mating

A completely recessive lethal with a mutation rate of  $10^{-5}$  in a random mating population will have an equilibrium frequency of

$$\hat{q} = \sqrt{\frac{u}{s}} = \sqrt{\frac{10^{-5}}{1}} = 3 \times 10^{-3}$$

By contrast, in a population with an  $F$  of 0.33 (50% selfing), a recessive lethal will have an equilibrium frequency 1/100 that in a random mating population:

$$\hat{q} \sim \frac{\sqrt{(F^2 + 4u/s)} - F}{2} = \frac{\sqrt{(0.33^2 + 4 \times 10^{-5}/1)} - 0.33}{2} = 3 \times 10^{-5}$$

For a partially recessive lethal allele with  $hs = 0.02$  ( $s = 1$ ,  $h = 0.02$ ), the equilibrium in a random mating population is

$$\hat{q} = \frac{u}{hs} = \frac{10^{-5}}{0.02} = 5 \times 10^{-4}$$

while for a population with an inbreeding coefficient of  $F = 0.33$ , the equilibrium frequency is about 1/17 that in random mating populations:

$$\hat{q} = \frac{u}{s(h+F)} = \frac{10^{-5}}{1(0.02+0.33)} = 2.86 \times 10^{-5}$$

For a highly inbred population, such as one with continual selfing, where  $F$  approaches 1, the equilibrium frequency is approximately

$$\hat{q} \sim \frac{u}{s} \sim 10^{-5}$$

Consequently, inbreeding reduces the equilibrium frequencies to 1/50 that for a partial recessive and to 1/300 that for a complete recessive in random mating populations.

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## Inbreeding in polyploids

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Inbreeding reduces heterozygosity more slowly in polyploids than in diploids

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Since many plants and some animals are polyploid, we need to consider the impact of inbreeding in them. To illustrate the principles involved we restrict consideration to autotetraploids. In tetraploids all gene copies must be identically homozygous for a complete recessive to be exposed. Thus, we expect that inbreeding will have a lesser impact in polyploids than in

diploids.

The pattern of segregation in autotetraploids is more complex than in diploids, as it depends upon the position of a locus relative to the centromere. Gametic output for two situations is given in [Table 12.6](#). For loci distant from the centromere, crossing over between the centromere and the locus makes it possible to produce AA gametes from Aaaa parents and aa gametes from AAAa parents, while these gametes are typically not produced for loci close to the centromere.

**Table 12.6** *Gametic output for different genotypes in an autotetraploid for loci close to, and distant from, the centromere*

Parent genotype	Gametic output	
	Distance from centromere	
	Close	Distant
AAAa	1AA:1Aa	15AA:12Aa:1aa
AAaa	1AA:4Aa:1aa	3AA:8Aa:3aa
Aaaa	1Aa:1aa	1AA:12Aa:15aa

Source: After Bever & Felber (1994).

The impact of selfing in an autotetraploid is shown in [Table 12.7](#). In contrast to a diploid, where selfing of an Aa heterozygote produces 25% aa recessive homozygotes, selfing of an AAaa autotetraploid produces only 2.8% or 4.6% aaaa recessive homozygotes depending on the position of the locus. Heterozygosity halves each generation with selfing in a diploid, but for an autotetraploid locus it becomes ~1/5th and ~1/6th of the current value per generation for loci distant and close to the centromere, respectively (Bever & Felber 1994). Consequently, inbreeding in tetraploids is anticipated to have a far lesser impact on reproductive fitness than in diploids ([Chapter 13](#)).

**Table 12.7** *Phenotypic ratios produced by selfing for different genotypes in an autotetraploid, for loci close to and distant from the centromere. One A allele is assumed to produce the dominant A phenotype*

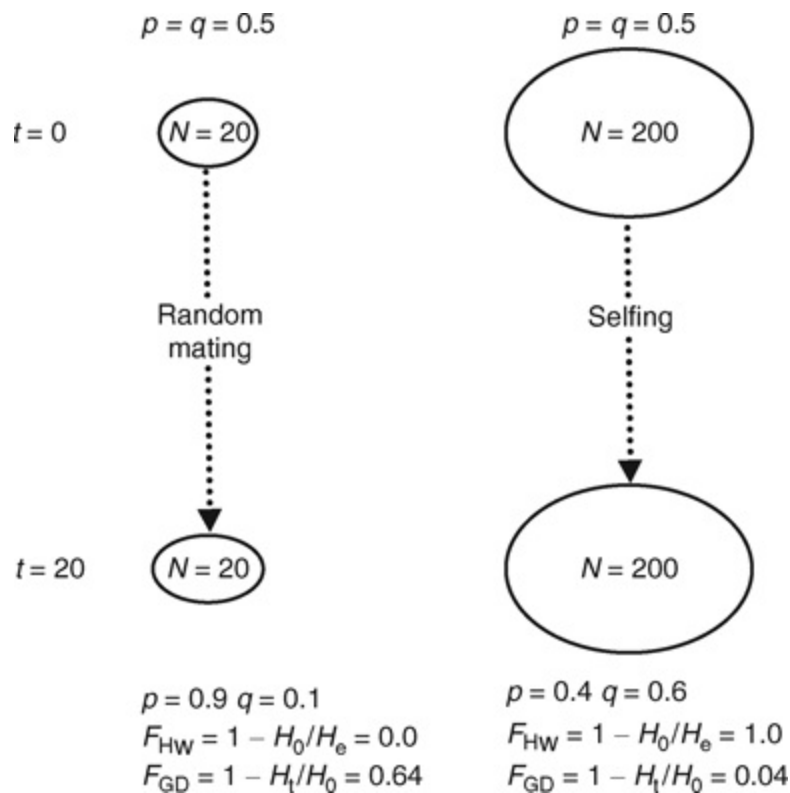
Parent genotype	Phenotypic ratios	
	Distance from centromere	
	Close	Distant
AAAA	All A	All A
AAAa	All A	783A: 1a
AAaa	35A: 1a	20.8A: 1a
Aaaa	3A: 1a	2.5A: 1a
aaaa	All a	All a

Source: After Allard (1960).

## Relationships between inbreeding, heterozygosity, genetic diversity and population size

The close relationship between inbreeding and loss of heterozygosity found in small random mating populations is usually not found in habitually inbreeding species

The connections between inbreeding, small population size and expected heterozygosity are a feature of random mating populations, but not of populations that naturally inbreed (Fig. 12.10).



**Fig. 12.10** Relationship between heterozygosity and inbreeding in a small random mating population and in a large selfing population. *In the small random mating population genotypes are in Hardy-Weinberg equilibrium ( $F_{HW} = 0$ ) and all inbreeding is due to drift ( $F_{GD} = 0.64$ ). In the large selfing population all individuals are homozygous ( $F_{HW} = 1.0$ ), but there is little inbreeding due to drift ( $F_{GD} = 0.04$ ).*

In a random mating population of stable size, loss of genetic diversity equals the inbreeding coefficient. Conversely, a large plant population that reproduces by selfing may have a high inbreeding coefficient in each individual and a very low observed heterozygosity, but high overall genetic diversity as alleles are distributed among, rather than within individuals. Loss of genetic diversity as measured by polymorphism and allelic diversity is due to sampling effects, rather than to inbreeding.

## Summary

1. Inbreeding is the production of offspring from mating of individuals

that are related by descent.

2. Inbreeding is an unavoidable consequence of small population size.
3. Inbreeding is of conservation concern as it reduces reproductive fitness in inbred populations and increases the risk of extinction.
4. Inbreeding is measured using the inbreeding coefficient ( $F$ ), the probability that an individual has two alleles at a locus that are identical by descent.
5. Inbreeding increases homozygosity and exposes deleterious recessive alleles.
6. Mutation–selection equilibria under inbreeding for recessive alleles of large effect are lower than under random mating (purging), but may show little difference for alleles of small effect.
7. Inbreeding increases homozygosity more slowly in polyploids than in diploids and is expected to have less impact on their fitness.

### **Further reading**

Charlesworth & Charlesworth (1987) Excellent review of theory and empirical issues relating to the impact of inbreeding.

Crow & Kimura (1970) *Introduction to Population Genetics Theory*. Advanced treatment of inbreeding theory.

Falconer & Mackay (1996) *Introduction to Quantitative Genetics*. Provides a clear treatment of inbreeding.

Malecot (1969) *The Mathematics of Heredity*. The classic work that defined inbreeding in probability terms.

Ralls & Ballou (1983) Highly influential study describing deleterious effects of inbreeding on captive wildlife.

### **Software**

SAS Proc Inbreed: Commercial software that includes procedures for calculating inbreeding coefficients from pedigrees. [www.sas.com/](http://www.sas.com/)



PMx: Free software for calculating inbreeding and demographic analyses on populations with pedigrees (Pollak *et al.* 2009). [www.vortex9.org/pmx.html](http://www.vortex9.org/pmx.html)

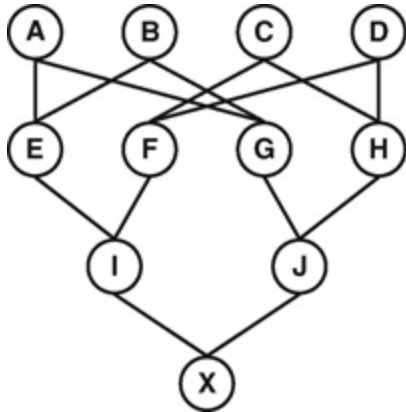
PEDSYS: A commercial database system for management of genetic, pedigree and demographic data. [www.sfbr.org/pedsys/pedsys.html](http://www.sfbr.org/pedsys/pedsys.html)

## Problems

- 12.1 Inbreeding coefficients. What is the inbreeding coefficient for the white tiger on the front of this chapter that resulted from a father–daughter mating?
- 12.2 Inbreeding in small populations. What is the average inbreeding coefficient for the Isle Royale gray wolf population with an effective size of 5 for 10 generations?
- 12.3 Inbreeding in fluctuating populations. Compute the inbreeding coefficient for a population of Mauritius kestrels that fluctuates in size as follows: 2, 100, 2, 100.
- 12.4 Genotype frequencies with inbreeding. Determine the genotype frequencies for the chondrodystrophy locus in California condors for progeny that would result if they could self (see [Table 12.3](#)).
- 12.5 Inbreeding and homozygosity. By what factor is the frequency of deleterious recessive homozygotes increased in children of (a) full-sib mating, and (b) first-cousin mating, compared to random mating when the deleterious allele has a frequency of 1%?
- 12.6 Recurrent inbreeding. What is the inbreeding coefficient for the first five generations using repeated full-sib mating in the naked mole rat?
- 12.7 Inbreeding and heterozygosity. What will be the frequency of heterozygotes at the *Mdh-2* locus with alleles F and S at frequencies of 0.4 and 0.6 in a population of Pacific yew with an inbreeding coefficient of 0.47 due to non-random mating (El-Kassaby & Yanchuk 1994)?
- 12.8 Effective inbreeding coefficient. Microsatellite heterozygosity in

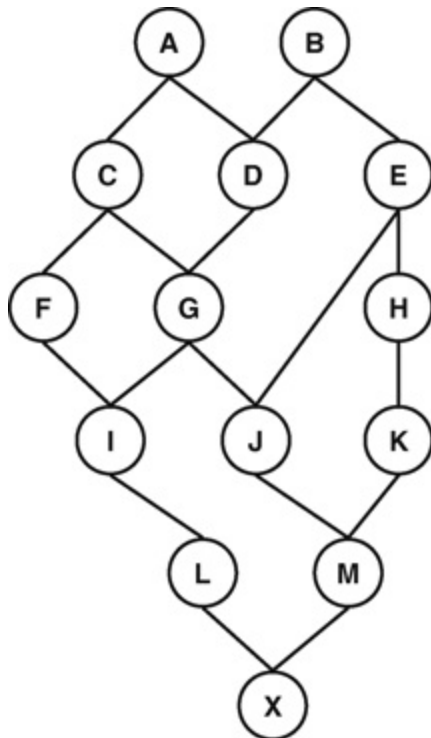
the black bears on the island of Newfoundland is 36%, and 79.2% on the Canadian mainland (Paetkau & Strobeck [1994](#)). What is the effective inbreeding coefficient for the Newfoundland population, relative to the mainland population?

- 12.9** Pedigree inbreeding. Determine the inbreeding coefficient of individual X in the pedigree shown in the margin (the results of a double first-cousin mating).



Problem 12.9

- 12.10** Pedigree inbreeding. What is the inbreeding coefficient for individual X in the pedigree shown in the margin (after Hedrick [1983](#))?



Problem 12.10

- 12.11** Pedigree inbreeding. What is the inbreeding coefficient for the Nigerian giraffe (individual X) in Paris Zoo ([Box 2.1](#))?
- 12.12** Deleterious effects of inbreeding. What is the probability that individuals resulting from selfing are homozygous for at least one lethal allele in an outbreeding plant species with 2000 loci that can mutate to produce lethals? Assume that deleterious alleles at each locus have equilibrium frequencies of  $5 \times 10^{-4}$ . Compare this with the probability for a random mating population.

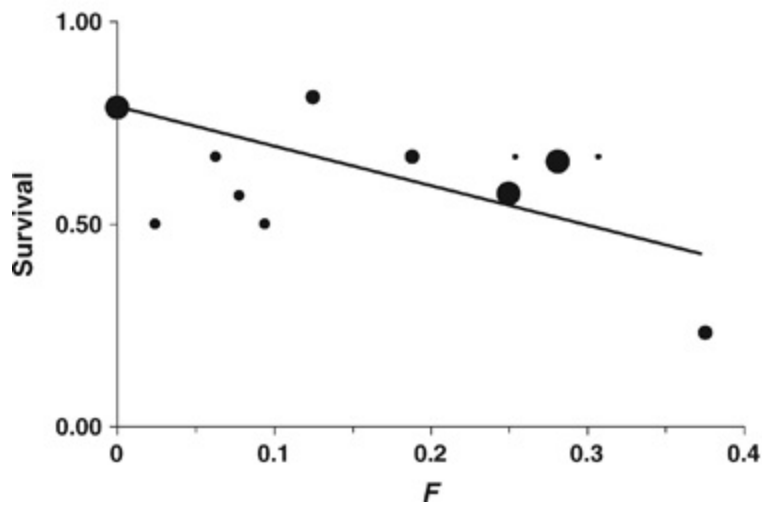
## Chapter 13 Inbreeding depression

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Inbreeding reduces reproductive output and survival in essentially all well-studied, naturally outbreeding species and, to a lesser extent, in selfing species. Outcrossing reverses these deleterious effects

### **Terms**

Dominance, epistasis, genetic rescue, heterosis, lethal equivalents, outcrossing



Inbreeding depression: survival of juvenile pygmy hippopotamus from Africa declines with increasing inbreeding ( $F$ )

## Inbreeding depression in naturally outbreeding species

Inbreeding in naturally outbreeding populations of animals and plants results in a decline in reproductive fitness, termed inbreeding depression

Darwin (1876) documented inbreeding depression in studies of 57 species of plants from 52 genera and 30 families (Table 13.1). Inbred plants were on average shorter, weighed less, flowered later and produced fewer seeds than outbred plants. On average, selfed plants showed a 41% reduction in seed production and a 13% decline in height. Not all species showed inbreeding depression for all characters studied, but virtually all cases showed inbreeding depression for most characters.

**Table 13.1** *Effects of inbreeding by self-fertilization (I) versus outcrossing (O) on several characters in 57 species of plants from Darwin's work*

Characters	Species	Experiments	O>I	O<I	Similar <sup>a</sup>	Difference (O – I)
Height	54	83	57	8	18	13%
Weight	8	11	8	1	2	
Flowering time	32	58	44 <sup>b</sup>	9	5	
Seed production	23	33	26	2	5	41%

<sup>a</sup> Darwin considered comparisons to be similar if they lay within 5% of each other.

<sup>b</sup> Outbreds flowered earlier than inbreds.

*Source:* Darwin (1876).

Subsequently, inbreeding depression has been documented in laboratory animals, domestic animals, outbreeding plants and humans (Lynch & Walsh 1998; Hedrick & Kalinowski 2000; Keller & Waller 2002; Frankham 2005)

(Table 13.2).

**Table 13.2 Inbreeding depression for different components of fitness in animals and plants due to a 25% increase in the inbreeding coefficient, expressed as % reduction in mean of inbreds compared to outbreds**

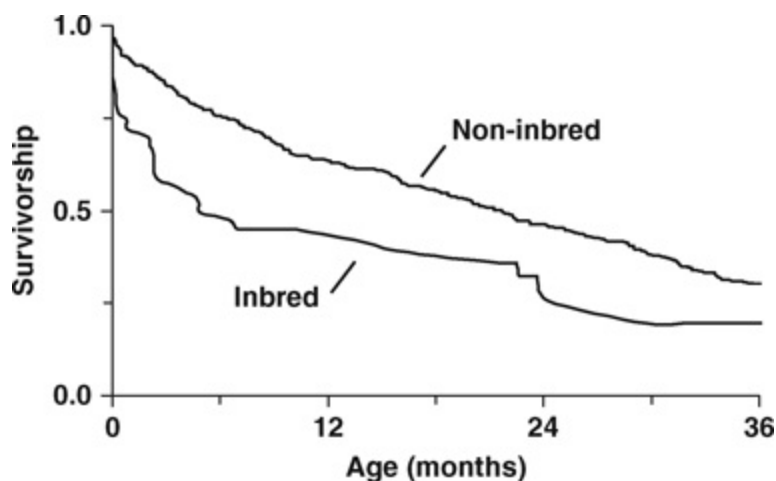
Species	Character	Inbreeding depression (%)	Species	Character	Inbreeding depression (%)
Humans	Height at age 10	4	<b>Wild species</b>		
	IQ score	11	Deer mice	Litter size	15
<b>Domesticated species</b>				Survival to weaning	8
Cattle	Milk yield	8	House mice (wild)	Litter size	10
Sheep	Fleece weight	14		Body weight	−10
	Body weight at 1 year	9		Nesting behaviour	10
			Japanese quail	Reproduction and survival	64
Species	Character	Inbreeding depression (%)	Species	Character	Inbreeding depression (%)
Pigs	Litter size	8		Fertility	21
	Body weight at 154 days	11		Survival 0–5 wks	10
Mice	Litter size	18		Body weight	4
	Body weight at 6 wks	2	Chukar partridges	Reproduction and survival	58
Chickens	Reproduction and survival	26		Egg production	16
	Egg production	10		Body weight	1
	Body weight	5	Rainbow trout	Hatchability	−10, 9, 14
Turkeys	Reproduction and survival	38		Fry survival	8, 11
	Egg production	10		Weight at 150 days	12
	Body weight	10	Zebrafish	Hatchability	89
Maize	Yield of seed (full-sib)	14		Survival to 30 days	43
	(selfing)	17		Length at 30 days	11
	Plant height (full-sib)	5	Channel catfish	Hatchability	−11
	(selfing)	6		Body weight at 4 wks	43
				Body weight at 12 wks	7

Sources: Abplanalp (1990); Falconer & Mackay (1996); Roff (1997).

## Inbreeding depression in the wild

Inbreeding depression occurs in the majority of wild populations investigated

There is now clear and irrefutable evidence for inbreeding depression in wild populations, despite earlier scepticism (Frankham 2005). Inbreeding depression in nature occurred for 90% of 157 valid data sets across 34 taxa (Crnokrak & Roff 1999). Significant inbreeding depression has also been reported in at least another 15 taxa (Frankham 2000b). For example, survival of inbred individuals is lower than that for outbred individuals in endangered golden lion tamarins in wild habitats in Brazil (Fig. 13.1). Moreover, hatching failure rates across 99 species of birds increases with the genetic similarity of parents (Spottiswoode & Møller 2004). The desert topminnow fish (Box 13.1) illustrates an excellent study of inbreeding depression in the wild.





**Fig. 13.1** Inbreeding depression for survival of endangered golden lion tamarins in natural habitats in Brazil (after Dietz *et al.* 2000).

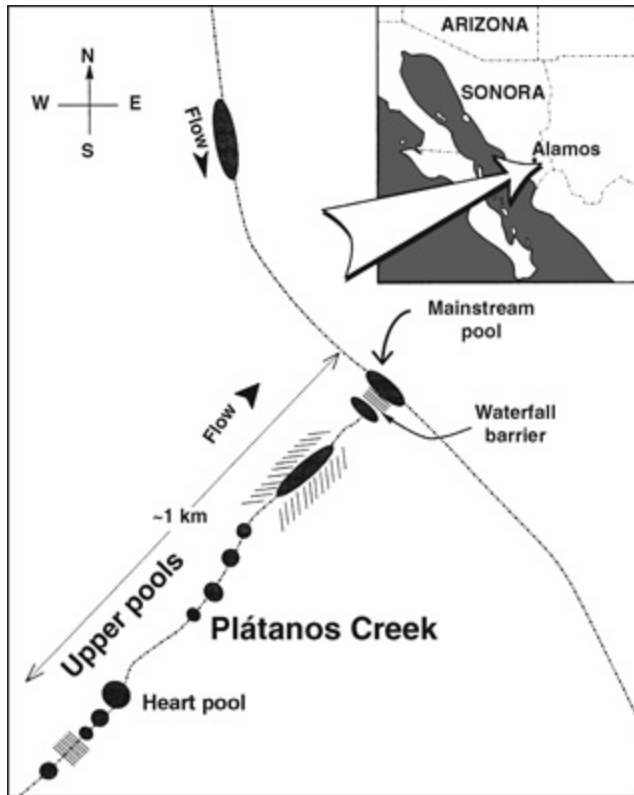
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**Box 13.1 Inbreeding depression in desert topminnow fish in the wild (after Vrijenhoek 1994)**

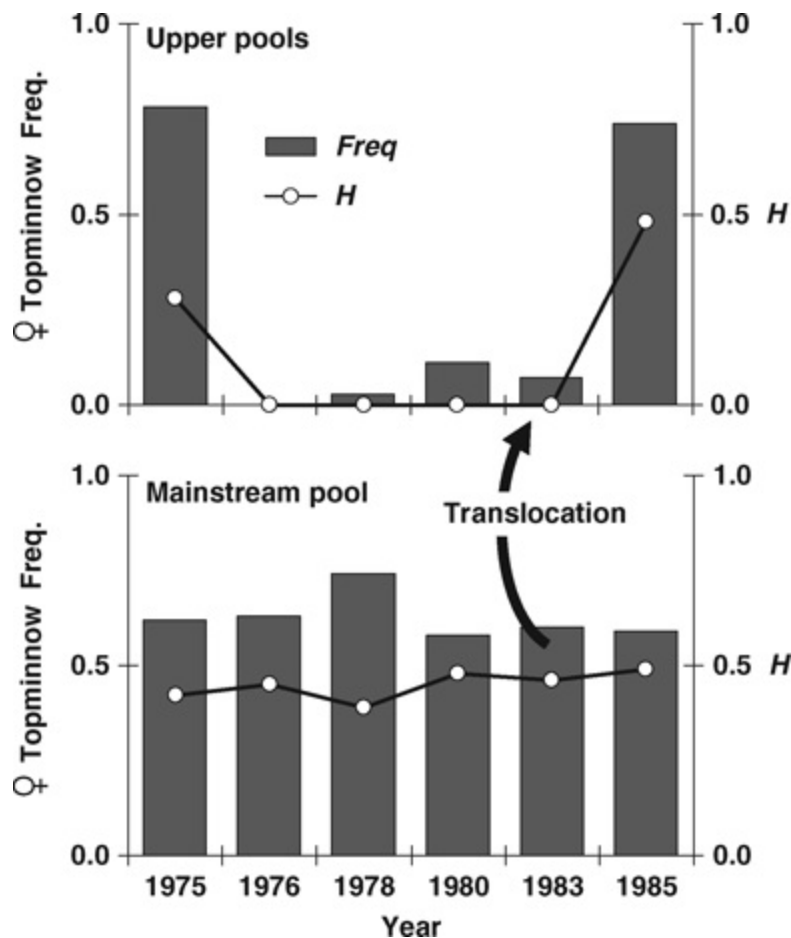
Populations of desert topminnow fish in Sonora, Mexico inhabit the upper pools in the Plátanos which were completely desiccated during a severe drought in 1975. By 1978, these pools had been recolonized, but were founded by a single gravid female. Prior to the drought, the topminnows coexisted with a clonal, parthenogenetic fish of the same genus, with the two forms representing 76% and 24%, respectively, of the fish density. After the populations were refounded and the sexual form had inbred, the sexual topminnows represented only 5% of the fish, indicating 93% inbreeding depression. No corresponding changes in relative abundance occurred in downstream populations where topminnows did not become inbred. The inbred population of topminnows showed spinal curvature and other deformities, and poorer resistance to low oxygen tension.



Desert topminnow fish



In 1983, 30 genetically variable female topminnows from the downstream population were exchanged with 30 inbred topminnow females from the upstream Heart Pool. By 1985, the sexual topminnow had re-established its numerical dominance over the clonal genotype and represented about 80% of the fish, i.e. adding outbred fish to the population reversed inbreeding depression.



Further, the load of a trematode parasite (the black spots on the fish illustrated above) is least in outbred fish and highest in inbred and clonal fish (Lively *et al.* 1990).

Evidence for inbreeding depression is so extensive that the default assumption for unstudied outbreeding species must be that they will exhibit inbreeding depression if they inbreed

Some studies have failed to detect inbreeding depression. However, these are usually very small studies or lack genetically verified paternities. For example, Rowley *et al.* (1993) found no inbreeding depression in splendid fairy wrens from Western Australia. Later paternity studies revealed that at least 64% of progeny were not fathered by the male to whom paternity had previously been attributed. Komdeur *et al.* (1998) detected no inbreeding depression in Seychelles warblers, but the study involved progeny from only 12 outbred and 17 inbred matings.

## Inbreeding depression due to small population size

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Inbreeding due to small population size results in inbreeding depression

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The inbreeding depression described above typically results from rapid inbreeding, such as self-fertilization and full-sib mating. However, inbreeding in threatened species typically occurs at a much slower rate in small closed populations that are approximately random mating (Chapter 12). Slow inbreeding has reduced population fitness in small approximately random mating populations of black-footed rock wallabies, euro kangaroos, greater prairie chickens, a snake, topminnow fish, fruit flies, house flies and plants (three species) (Frankham 1995a; Heschel & Paige 1995; Madsen *et al.* 1996; Fischer & Matthies 1998; Westemeier *et al.* 1998; Bryant *et al.* 1999; M. D. B. Eldridge *et al.* 1999, pers. comm.). For example, Box 13.2 details inbreeding depression for litter size and abnormal offspring in a small Swedish population of adders.

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### **Box 13.2 Inbreeding depression in a small isolated population of adders in Sweden (Madsen et al. 1996, 2004)**

In Sweden, a small isolated population of adders, with fewer than 40 individuals, has been separated from the main distribution of the snake for at least a century. Allozyme variability and DNA fingerprints confirmed that it had low levels of genetic diversity, and so was inbred, relative to the main population.



Swedish adder

The small population showed evidence of inbreeding depression. It displayed lower litter size and more abnormal offspring than in the larger population. Different environmental conditions were ruled out as an explanation for the high frequency of abnormal offspring, as the progeny of an introduced male from the large population, when mated to females from the small population, exhibited a reduced frequency of abnormalities.

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## **Inbreeding, population viability and extinction**

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Inbreeding increases the risk of extinction for most species, under a wide range of circumstances

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Since inbreeding reduces reproductive and survival rates, it is expected to increase the risk of extinction. This occurred in captive populations of mice and fruit flies, where other causes of extinction were controlled (Frankham 1995b; Fig. 2.1). Extinctions do not commence until intermediate levels of inbreeding, but this lag does not indicate that modest levels of inbreeding are benign. Population growth rates must become negative for extinctions to occur, meaning that inbreeding must reduce reproduction and survival to below replacement levels. For example, the number of progeny in fruit flies must drop from over 100 per pair to below two for extinctions to occur. Since there has been scepticism about the effects of inbreeding, it is no surprise that there is also scepticism about the relative importance of genetic factors as a cause of extinction in wild populations. Much of this doubt was due to a lack of evidence directly identifying genetic factors as common causes of extinction. However, there is now substantial evidence that inbreeding increases extinction risk in wild animal and plant populations (Frankham 2005; Chapter 2), namely:

- direct evidence of inbreeding increasing extinction risk of butterfly and plant populations
- computer projections indicate that inbreeding increases extinction risks under a wide range of realistic circumstances (Liberg *et al.* 2005; O'Grady *et al.* 2006)
- Circumstantial evidence that inbreeding may contribute to the extinction proneness of island populations (Frankham 1998; Groombridge 2007).

Inbreeding in a closed population operates in a ratchet fashion not found for other stochastic effects (demographic and environmental stochasticity and catastrophes). Once a closed population has become inbred, it retains this, even if population size recovers. Further, each additional population size reduction increases inbreeding, akin to a ratchet clicking one step further.

## **Inbreeding and population viability**

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The effects of inbreeding on population viability are complex, but they will usually be deleterious in the long term

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Scepticism has been expressed about the significance of inbreeding to population viability (Caro & Laurenson 1994; Caughley & Gunn 1996). Reference is often made to highly inbred populations with no apparent inbreeding depression. For example, a number of small, presumably inbred, island populations persist, including Chatham Island black robin, Hawaiian crow, Mauritius kestrel and Seychelles robin (Dhondt & Mattysen 1993; Craig 1994; Box 8.1). Further, several bottlenecked populations, including the northern elephant seal, have recovered without apparent ill effects (Box 8.2). Since there are typically no non-inbred controls, it is not possible to evaluate the effects of inbreeding in these populations. Further, they represent a selected sample which ignores populations that have gone extinct (Laikre *et al.* 1997). Two studies incorporating controls have both found adverse effects of small population size on fitness. Native and introduced New Zealand birds that have suffered bottlenecks have higher hatching failures than non-bottlenecked populations (Briskie & Mackintosh 2004). Further, of fruit fly populations that survived to an  $F = 0.8$ , all had lower fitness than their non-inbred base population (Reed *et al.* 2003a).

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Inbreeding depression does not necessarily cause declines in population size

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Inbreeding interacts with basic parameters of population viability, population growth rate and variation in population size. While these interactions may be complex, inbreeding in naturally outbreeding species will almost always be deleterious to closed populations in the long term, even if the impacts on population size are not evident initially (O'Grady *et al.* 2006). For example, a healthy outbred population (with a positive  $r$ ) beginning at a small size and having a carrying capacity ( $K$ ) due to limited habitat, will rapidly grow to  $K$  individuals (Box 13.3). A mildly inbred population will also grow to size  $K$ , but more slowly. A moderately inbred population will grow to the same carrying capacity even more slowly. However, a highly inbred population with a negative growth rate will decline to extinction. Whilst the first three populations reach a similar  $K$ , they have quite different capacities to recover from population catastrophes, or to absorb new impacts from introduced pests, parasites, diseases or predators. In all cases, inbred populations will be inferior to outbred populations.

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### **Box 13.3 Impact of inbreeding on population viability**

The effects of inbreeding on population growth can be illustrated using equations of population growth from ecology (see Gotelli 2008, or any modern ecology textbook). A small population of size  $N$  in a constant environment with a large amount of available habitat is expected to show exponential growth. The rate of growth of population size ( $dN$ ) per increment of time ( $dt$ ) depends on the difference between the birth ( $b$ ) and death rates,  $r$ , and the population size  $N$ , as follows:

$$\frac{dN}{dt} = (b - d)N = rN$$

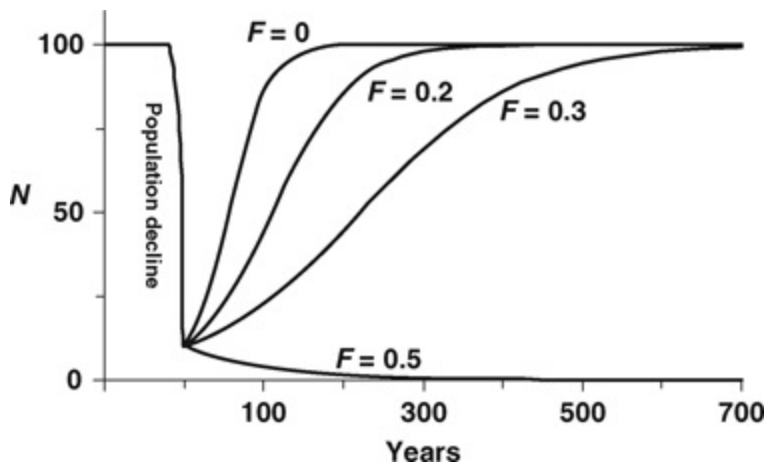
and the population size at time  $t$ ,  $N_t$ , is

$$N_t = N_0 e^{rt}$$



Let us consider a non-inbred population that has a growth rate  $r$ , of 0.04. Inbreeding is expected to reduce  $r$  by  $\sim 25\%$  per 10% increase in  $F$  (Frankel & Soulé 1981). The  $r$  for a mildly inbred population with  $F$  of 20% would be  $\sim 0.04 (1 - 2.5 \times 0.2) = 0.02$  (i.e. a 50% decrease), while  $r$  for a moderately inbred population ( $F = 0.3$ ) would be  $\sim 0.01$ . Finally, a highly inbred population with an  $F$  of 0.5 would have a negative growth rate of  $\sim -0.01$ .

As populations usually exist in a habitat with a limited carrying capacity, they typically show logistic population growth (see figure below). The outbred and the mildly and moderately inbred populations grow to the same carrying capacity  $K$ , i.e. they will eventually reach the same population sizes. Conversely, the highly inbred population declines towards extinction. Thus, populations exhibiting inbreeding depression may have the same sizes ( $K$ ) as related non-inbred populations. However, inbred populations take longer to reach the carrying capacity.



Outbred and mildly and moderately inbred populations will take different times to recover from catastrophes. For example, the approximate time for a population to recover its original size of  $K$  following a catastrophe resulting in a 90% reduction in population size ( $N_t = 10 N_0$ ) can be determined by rearranging the second equation, as follows:

$$\frac{N_t}{N_0} = 10 = e^{rt}$$

taking natural logarithms and rearranging this yields:

$$t = \frac{\ln(10)}{r}$$

By substituting for  $r$ , we obtain 57.6 years for the outbred population to recover its original size, 115 years for the mildly inbred population, and 230 years for the moderately inbred population. The highly inbred population ( $F = 0.5$ ) with  $r = -0.01$  will not recover and will become extinct. While the mildly and moderately inbred populations will eventually recover to their original size, another catastrophe may strike them before they have done so.

Adverse effects of inbreeding on population growth rates have been described in eastern mosquito fish, red flour beetles and two species of wolf spiders, and probably occur in almost all naturally outbreeding species (O'Grady *et al.* 2006; Reed *et al.* 2007). Populations of mosquito fish founded from brother–sister pairs exhibited 56% lower growth in numbers than populations founded from unrelated pairs (Leberg 1990a). Strong reductions in population growth have been observed in inbred flour beetle populations and adverse effects were detected at an  $F$  of only 10% (McCauley & Wade 1981).

The northern elephant seal population grew in size despite inbreeding resulting from a bottleneck of 20–30 individuals. It has recovered to over 175 000 (Box 8.2). The reasons for this are two-fold. First, the decline in numbers was due to over-hunting, now stopped by legislative protection. Second, the inbreeding did not result in a negative population growth rate.

## Characteristics of inbreeding depression

All components of reproductive fitness are subject to inbreeding

depression

Inbreeding has been shown to adversely affect all aspects of reproductive fitness, including offspring numbers, juvenile survival, longevity, interbirth interval, mating ability, sperm quantity and quality, maternal ability, competitive ability, developmental time, immune response and disease resistance in animals (Reid *et al.* 2003; Spielman *et al.* 2004b; Whiteman *et al.* 2006) (see Table 13.2). For example, in old-field mice, inbred dams were less likely to breed and those that did were less likely to have a second litter (Lacy *et al.* 1996). Their litters were smaller, survival of inbred offspring from birth to weaning was lower (69% vs. 93%), and mass of inbred pups at weaning and overall mass of progeny produced were reduced, when compared to outbred dams. Sperm abnormalities were higher, and sperm motility lower, in small inbred populations of lions and Florida panthers than in related large populations (Wildt 1996). In plants, pollen quantity, number of ovules, amount of seed, germination rate, growth rates and competitive abilities all exhibit inbreeding depression (Tables 13.1 and 13.2).

Characters most closely related to reproductive fitness show greater inbreeding depression than those only peripherally related to fitness

Seed production/grain yield show greater inbreeding depression than height in plants (Tables 13.1 and 13.2). Similarly, reproduction, survival and litter size typically show more inbreeding depression than body size in animals

(Mousseau & Roff [1987](#); Roff & Mousseau [1987](#)) ([Table 13.2](#)).

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Inbreeding depression is greater for total fitness than for its components

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Greater inbreeding depression for overall fitness, compared to its components, has been found for old-field mice, house mice, chickens, turkeys, Japanese quail, chukar partridges and song sparrows (Beilharz [1982](#); Abplanalp [1990](#); Lacy *et al.* [1996](#); Keller [1998](#); Meagher *et al.* [2000](#); [Table 13.2](#)). Impacts of inbreeding on total fitness are often very large. For example, Meagher *et al.* ([2000](#)) found a 57% reduction in total fitness in mice due to full-sib inbreeding vs. outbreeding, under semi-wild conditions. In wild song sparrows, Keller ([1998](#)) reported a 79% reduction in offspring numbers in the progeny of full-sibs compared to the progeny of unrelated individuals.

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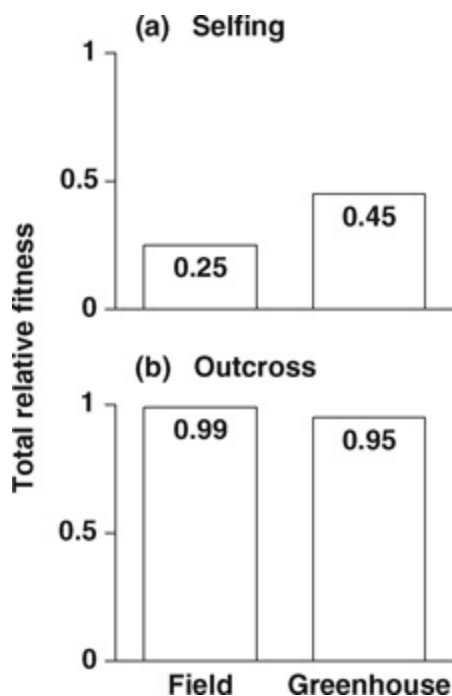
Inbreeding depression is greater under more stressful conditions

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Inbreeding depression was, on average, 69% greater in stressful than in benign environments, in a meta-analysis of 34 studies (Armbruster & Reed [2005](#)). Inbreeding depression in plants is typically greater in the field than in

greenhouses. For example Dudash (1990) found that selfed progeny of the rose pink plant exhibited 75% inbreeding depression in the field, but only 53% in the greenhouse (Fig. 13.2). Inbreeding reduced survival in Soay sheep under conditions of high population densities, due to gastrointestinal nematodes, but the effect was not found at low densities, nor in sheep cleared of nematodes (Coltman *et al.* 1999). Further, experiments with fruit flies have shown elevated extinction rates of inbred populations under stressful environments (Bijlsma *et al.* 2000; Reed *et al.* 2002).



**Fig. 13.2** Inbreeding depression in the rose pink plant is greater under more stressful conditions (after Dudash 1990). Relative fitnesses in propagules resulting from self-pollination, and cross-pollination between populations, when grown in the greenhouse versus field conditions.

Inbreeding depression is typically much greater in the wild than in captivity

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Inbreeding depression for mammals is 6.9 times higher in the wild than in captivity, in line with greater impacts in more stressful wild versus more benign captive conditions (Crnokrak & Roff [1999](#)).

## **Variability in inbreeding depression**

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Inbreeding depression has a large stochastic element

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Inbreeding depression is expected to have a large stochastic element, since it depends on the frequency of homozygotes for deleterious alleles. Due to genetic drift, the same deleterious allele may be absent in one population, but present at moderate frequency in another. Further, different loci will, by chance, become homozygous in different individuals. Consequently, different species, populations, families and individuals will differ in their complement of deleterious alleles and in their homozygosity at different loci. They will thus show differences in their susceptibility to inbreeding depression. Since many loci affect reproductive fitness, it is highly improbable that fixation of deleterious alleles will be avoided at all loci. This accounts for the ubiquitous, but highly variable, nature of inbreeding depression.

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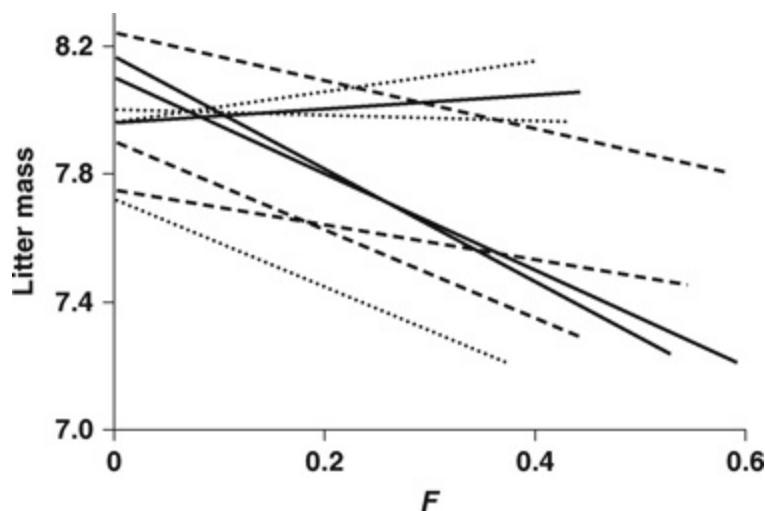
Species, populations and families differ in inbreeding depression

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Empirical studies exhibit variable inbreeding depression across species, populations, families and individuals, as predicted. Ralls and Ballou (1983) found substantial variation in inbreeding effects across 44 different mammal species (Fig. 12.1). Variation in inbreeding depression among lineages, within species has been reported in old-field mice, dairy cattle, fruit flies and flour beetles (Reed *et al.* 2002; Armbruster & Reed 2005), and is expected in all naturally outbreeding species. Inbreeding depression varies among three sub-species of old-field mice, and among three samples within each sub-species (Fig. 13.3). Further, Kärkkäinen *et al.* (1996) reported geographic variation in inbreeding depression in the Scots pine.



Old-Field mouse



**Fig. 13.3** Differences in inbreeding depression among three sub-species of old-field mice (solid, dashed and dotted lines) and of three samples within each sub-species for total mass of progeny weaned per pair (after Lacy *et al.* 1996). Regression lines relating fitness and inbreeding coefficient for each

replicate of each sub-species are plotted.

Species and populations vary in the components of fitness that are affected by inbreeding. Captive populations of Mexican and red wolf do not exhibit inbreeding depression for juvenile survival, but show inbreeding depression in adult survival (Kalinowski *et al.* 1999; Wilcken 2002). Similarly, replicates differed in the fitness components affected by inbreeding in the study of old-field mice (Lacy *et al.* 1996).

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All major taxa of plants and animals show inbreeding depression in naturally outbreeding species

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Little variation in susceptibility to inbreeding is evident among major taxonomic groups, in contrast to the differences within species. No differences were found among mammalian orders in inbreeding depression for juvenile survival (Ralls *et al.* 1988). Inbreeding depression was very similar across birds, mammals, poikilotherms and plants (Crnokrak & Roff 1999).

Gymnosperms and angiosperms do differ in inbreeding depression (64% vs. 39% reduction in fitness traits due to selfing: Husband & Schemske 1996). This difference is due to the combined effects of higher mutation rates in long-lived conifers than in short-lived angiosperms (Lynch & Walsh 1998), higher levels of selfing in angiosperms than gymnosperms, and the lower rates of polyploidy in gymnosperms than angiosperms (see below) (Scofield & Schultz 2006; Chapter 16).



## Inbreeding depression in species that regularly inbreed

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Species that naturally inbreed exhibit inbreeding depression, but its magnitude is generally less than that found in natural outbreeders

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Naturally inbreeding species are expected to suffer less inbreeding depression than outbreeders, since selection against deleterious recessives is generally more effective in inbreeders ([Chapter 12](#)). For example, the reductions in fitness due to self-fertilization in plants were 23% for selfers and 53% for outbreeders, and inbreeding depression and selfing rate were negatively correlated (Husband & Schemske [1996](#)).

Lower inbreeding depression in species that self could be due either to prior inbreeding, or to natural selection having previously purged deleterious alleles. Inbreeding depression due to one generation of inbreeding depends on the increase in inbreeding coefficient ( $\Delta F$ ) in that generation. For selfing from an outbred population,  $\Delta F$  is  $\frac{1}{2}$ , while it is only  $\frac{1}{4}$  for selfing from a plant that was itself the product of selfing in the previous generation. It only requires that the selfing species analysed by Husband & Schemske ([1996](#)) were subjected to only one prior generation of selfing to explain most of the difference in inbreeding depression between them and the outbreeding species. However, purging is also implicated (see below).

## Genetic basis of inbreeding depression

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The magnitude of inbreeding depression depends upon heterozygosity for deleterious alleles ( $2pq$ ), the dominance of alleles ( $d$ ) and the amount of inbreeding ( $F$ )

To determine the genetic basis of inbreeding depression, we develop a simple model for the effects of inbreeding on the mean of a character (Table 13.3). We consider a population with an inbreeding coefficient of  $F$  and a locus with two alleles  $A_1$  and  $A_2$ , at frequencies of  $p$  and  $q$ , and no selection. If the genotypes  $A_1A_1$ ,  $A_1A_2$  and  $A_2A_2$  have genotypic values of  $a$ ,  $d$ , and  $-a$  (Fig. 5.2), then the mean of the inbred population is:

$$M_F = M_0 - 2pqdF$$

Consequently, the inbreeding depression ( $\delta$ ) is:

$$\delta = 2pqdF \tag{13.1}$$

Thus, inbreeding depression depends upon the inbreeding coefficient, and the heterozygosity and dominance deviation ( $d$ ) for deleterious alleles. This equation leads us to expect a linear relationship between inbreeding depression and  $F$ , an issue we discuss below.

**Table 13.3** *Impact of inbreeding on the mean of a population: single locus model (see Fig. 5.2)*

Genotype	Value	Genotype frequencies		Genotype frequency x value	
		Random mating	Inbred	Random mating	Inbred
$A_1A_1$	$a$	$p^2$	$p^2 + Fpq$	$p^2a$	$p^2a + Fpqa$
$A_1A_2$	$d$	$2pq$	$2pq(1 - F)$	$2pqd$	$2pqd(1 - F)$
$A_2A_2$	$-a$	$q^2$	$q^2 + Fpq$	$-q^2a$	$-q^2a - Fpqa$
		Means		$M_0 = a(p - q) + 2pqd$	$a(p - q) + 2pqd - 2dpqF$ $M_F = M_0 - 2dpqF$

Inbreeding depression only occurs when there is dominance, or overdominance

A locus will only contribute to inbreeding depression if  $d > 0$ . Deleterious alleles must be partially, or completely recessive (and favourable alleles dominant, or partially so), or show overdominance (see [Fig. 5.3](#)) to contribute to inbreeding depression as illustrated in Example 13.1. While both mechanisms cause inbreeding depression, they respond differently to natural selection. With dominance, selection can reduce the frequency of deleterious alleles, but it cannot do this with overdominance (see following section on ‘Purging’). Evidence indicates that dominance of favourable alleles makes a substantially greater contribution to inbreeding depression than overdominance (Charlesworth & Charlesworth [1999](#); Swanson-Wagner *et al.* [2006](#)).

### Example 13.1 Effect of different levels of dominance on

## inbreeding depression

The values of  $d$  (dominance deviations for the heterozygotes) for additive, dominant and overdominant loci are 0,  $a$  and  $> a$ , respectively (Fig. 5.3). If we consider a locus with two alleles at frequencies of 0.99 for  $A_1$  and 0.01 for deleterious  $A_2$ , with an effect of 10% on survival ( $a = 0.05$ ), then the inbreeding depression for a full-sib mating ( $F = 0.25$ ) will be as follows.

For a locus with complete dominance ( $d = a = 0.05$ ), inbreeding depression ( $\delta$ )

$$\delta = 2pqdF = 2 \times 0.01 \times 0.99 \times 0.05 \times 0.25 = 2.47 \times 10^{-4}$$

An equivalent locus exhibiting partial dominance ( $d = 0.05/2 = 0.025$ ) will express half the inbreeding depression shown by a fully dominant locus.

Inbreeding depression for a locus with overdominance is expected to be higher as the allele frequencies will typically be more intermediate, yielding a higher value for  $2pq$ . Further, the value of  $d$  ( $> a$ ) is typically greater than that for the dominant cases.

Conversely, for a locus with additive effects ( $d = 0$ ), inbreeding depression

$$\delta = 2pqdF = 2 \times 0.01 \times 0.99 \times 0 \times 0.25 = 0$$

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Effects of inbreeding depression accumulate over all loci polymorphic for deleterious alleles

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If the combined genotypic values are given by the sum of the effects of the individual loci, the population mean is:

$$\begin{aligned} M_F &= \sum_{i=1}^{\# \text{ loci}} [a_i(p_i - q_i) + 2d_i p_i q_i (1 - F)] \\ &= M_0 - 2F \sum_{i=1}^{\# \text{ loci}} d_i p_i q_i \end{aligned}$$

Consequently, the cumulative inbreeding depression over loci is

$$\delta = 2F \sum_{i=1}^{\# \text{ loci}} d_i p_i q_i \quad (13.2)$$

Thus, inbreeding depression depends on  $F$ , the number of loci polymorphic for deleterious alleles, the dominance of the alleles and their frequencies. For inbreeding to change the mean, dominance ( $d$ ) must be directional, i.e. deleterious alleles must be partially to completely recessive. Such directional dominance is expected and observed ([Table 7.4](#)).

The cumulative effects of multiple loci segregating for lethals alone can account for high levels of inbreeding depression, as we saw in [Box 12.2](#) using realistic numbers and effects of loci. In reality, inbreeding depression derives from a combination of alleles with effects ranging from lethal to mildly deleterious. In fruit flies (the only genus for which we have reasonable data), inbreeding depression is about equally attributable to lethals and to deleterious alleles of small effect (Simmons & Crow [1977](#); Lynch & Walsh [1998](#)).

## Linearity of inbreeding depression with F

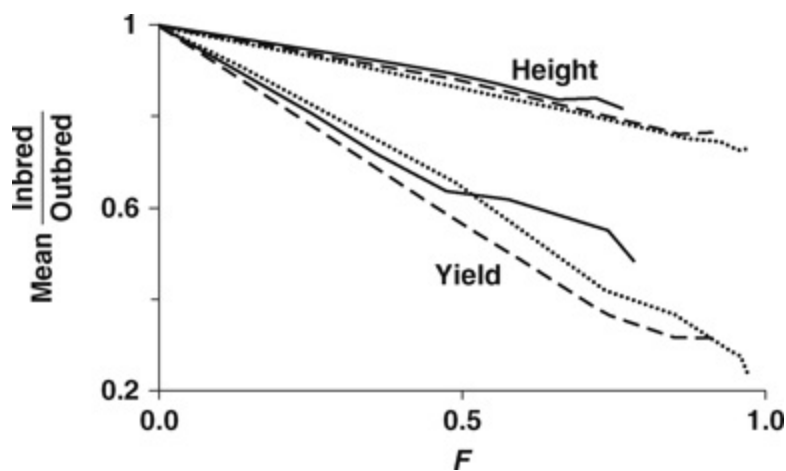
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Simple theory predicts a linear relationship between the mean value of a

fitness character and the inbreeding coefficient, and empirical data generally supports this prediction

Inbreeding depression is expected to be linearly related to the inbreeding coefficient  $F$ , if effects of different loci combine additively (Equation 13.2). For survival, where we multiply the fitness effects of different loci, the logarithm of survival should show a linear relationship with inbreeding, as discussed below. More complex models of inbreeding depression involving interactions among loci (epistasis) may result in additional, curvilinear effects, depending on  $F^2$ .

Available data generally indicate an approximately linear relationship between mean fitness and the inbreeding coefficient (Lynch & Walsh 1998). For example, grain yield and height in maize show essentially linear declines with inbreeding (Fig. 13.4). However, a few cases show non-linear relationships between mean and  $F$ .



**Fig. 13.4** Linearity of inbreeding depression on height and grain yield in maize (after Falconer & Mackay 1996, based on data from Hallauer & Sears (dotted lines) and Cornelius & Dudley (continuous and dashed lines)). The dotted and dashed lines refer to consecutive selfing and the solid line to full-sib mating. *The declines are approximately linearly related to  $F$ , and more severe for yield than height.*

## Effects of ploidy

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Ploidy has a major effect on inbreeding depression; haploids lack inbreeding depression, while polyploids may exhibit less than diploids

Inbreeding depression differs for haploids, diploids and tetraploids, as it is directly related to the expression of deleterious alleles, and to the frequency of heterozygotes. As there are no hidden deleterious recessive alleles, it is absent in haploids. The most plausible models lead us to expect less inbreeding depression in tetraploids than in diploids. The rate of fixation is slower in tetraploids than diploids for the same amount of inbreeding, while genetic loads for partially recessive alleles are expected to be similar in diploids and tetraploids ([Chapter 12](#)). If overdominance was a major cause of inbreeding depression (unlikely), then inbreeding depression is predicted to be greater for tetraploids than for diploids (Husband & Schemske [1997](#)). Inbreeding depression for cumulative fitness due to selfing was greater in diploid (0.95) than in tetraploid (0.68) forms of fireweed (Husband & Schemske [1997](#)).

## Purging

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Inbreeding depression may be reduced, or purged, by selection against deleterious alleles, but it is unlikely to be eliminated

Purging has been documented in many species, including plants, mice, birds and fruit flies (Byers & Waller [1999](#); Crnokrak & Barrett [2002](#); Boakes *et al.* [2007](#); Leberg & Firmin [2008](#)).

Four factors affect purging:

- the genetic basis of inbreeding depression (dominance versus overdominance)
- natural selection reducing the frequency of deleterious alleles
- the effects of prior inbreeding in reducing the amount of new inbreeding
- the impact of new mutations.

These issues are now discussed and their impacts evaluated.

Rare deleterious recessive alleles are exposed by inbreeding and can therefore be more effectively removed by natural selection. However, purging does not operate on that component of genetic load due to overdominant loci, as selection continues to favour heterozygotes (Charlesworth & Charlesworth [1987](#)).

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New deleterious mutations are being added to populations each generation

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One reason for the persistence of inbreeding depression in small populations, and those which habitually inbreed, is the steady recurrence of deleterious mutations. The effect of continued small population size (or recurrent



inbreeding) on inbreeding depression depends on its effect on mutation–selection equilibria. As we saw in [Chapter 8](#) ([Fig. 8.9](#)), the effect of population size on mutation–selection equilibria for partial recessives (probably the predominant mutations contributing to inbreeding depression) is modest. Thus, a prior history of small size (or recurrent inbreeding) may reduce subsequent inbreeding depression, but is unlikely to eliminate it.

## Inbreeding and selection

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Selection against deleterious recessives is more effective in partially inbred populations, but its impact depends upon the size of effect of the alleles and on the population size

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The impact of selection in large, partially inbreeding populations can be determined by modelling selection against a deleterious partially recessive allele (frequency  $q$ ) with relative fitnesses  $A_1A_1 = 1$ ,  $A_1A_2 = 1 - hs$  and  $A_2A_2 = 1 - s$ . The decrease in the frequency of the deleterious  $A_2$  allele is then:

$$\Delta q = \Delta q_{\text{outbred}} + spqF(1 - 2h) \quad (13.3)$$

where  $\Delta q_{\text{outbred}}$  is the change in allele frequency expected due to selection in an outbred population and the term on the right is the additional change due to inbreeding. Thus, selection against a deleterious partial recessive is more effective in a large inbred population than in a large outbred population, by an amount that depends on the inbreeding level ( $F$ ), the selection coefficient ( $s$ ), the allele frequencies and the dominance of the allele ( $h$ ).

In small, partially inbreeding populations, selection is less effective, and

only deleterious alleles of large effect will be effectively purged (Hedrick 1994). Some deleterious alleles of small effect are likely to be fixed due to genetic drift in small populations (Chapter 8). Thus, purging regimes (i.e. intentional inbreeding to remove deleterious alleles) can actually reduce reproductive fitness, rather than increasing it. Overall levels of genetic diversity for rare deleterious alleles in small, compared to large populations are expected to show:

- lower frequencies of deleterious alleles of large effect, due to purging (fate determined by selection)
- slightly lower frequencies of partially recessive deleterious alleles of moderate effect, due to mutation–selection–drift balance
- a higher frequency of mildly deleterious alleles as they are effectively neutral in small populations and are therefore in mutation–drift equilibrium, rather than mutation–selection–drift equilibrium.

In fruit flies, deleterious alleles of large effect cause about half of the inbreeding depression and alleles of smaller effect contribute the remaining half (Simmons & Crow 1977). However, the proportions falling into the remaining categories are unclear and are dependent upon the population size. Overall, genetic diversity for deleterious alleles is likely to be lower in smaller than in larger populations, but the differences may be modest, rather than very large.

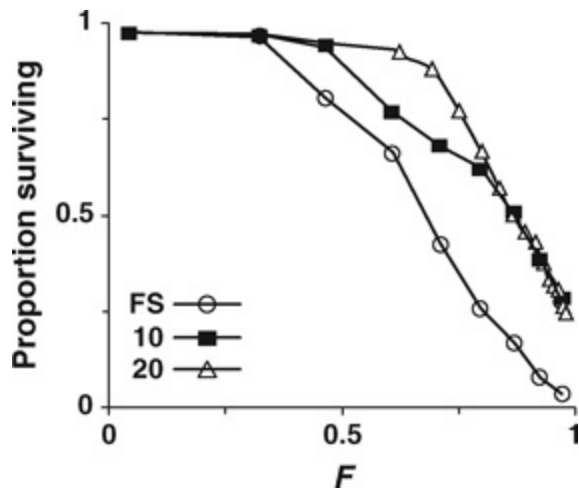
## Relationship of inbreeding depression to rate of inbreeding

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Slower inbreeding generally causes less inbreeding depression than an equivalent amount of rapid inbreeding, but the difference is often small

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Much of the inbreeding in endangered species in nature results from the cumulative impacts of small population size, i.e. slow inbreeding. Since the opportunities for natural selection to act are greater with slower inbreeding, the effects of a similar amount of inbreeding are predicted to be reduced with slower inbreeding. This prediction has proved to be generally correct, as shown in Fig. 13.5, although the effects are usually relatively small.



**Fig. 13.5** Relationship between inbreeding ( $F$ ) and proportion of replicate populations surviving for populations maintained using brother–sister mating (full-sib, FS) or genetically effective sizes of 10 and 20 (Reed *et al.* 2003a). The latter two treatments correspond to wild populations of about 100 and 200 adults per generation, typical of many endangered species. *The proportions of populations going extinct rises with inbreeding, no matter what the rate of inbreeding, but there is a greater extinction risk with the fastest inbreeding.*

## Fitness rebounds following bottlenecks

Reproductive fitness usually declines as a consequence of severe population bottlenecks, but may partially recover due to natural selection removing deleterious alleles

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As a result of a bottleneck, some deleterious alleles are fixed, some are lost and most change in frequency. Those that are fixed cause a reduction in the mean fitness of the population and this contribution remains essentially constant in subsequent generations. Deleterious alleles that have been increased in frequency due to the bottleneck will subsequently decrease in frequency due to natural selection, especially if they have large effects and post-bottleneck populations are large. Thus fitness may rebound over generations following a bottleneck.

Partial recovery of fitness following severe bottlenecks has been reported in house flies, butterflies and fruit flies (Bryant *et al.* 1990; Saccheri *et al.* 1996; Fowler & Whitlock 1999; Crnokrak & Barrett 2002). In fruit flies, there was 28% reduction in the mean fitness at generation 3 of populations due to single-pair bottlenecks, while 21% depression remained at generation 20. Further, the recovery may only occur in one environment. Inbred fruit fly populations that had been artificially purged showed depressed fitness in a different, stressful environment (Bijlsma *et al.* 1999).

### **Inbreeding depression in species/populations with historically small populations**

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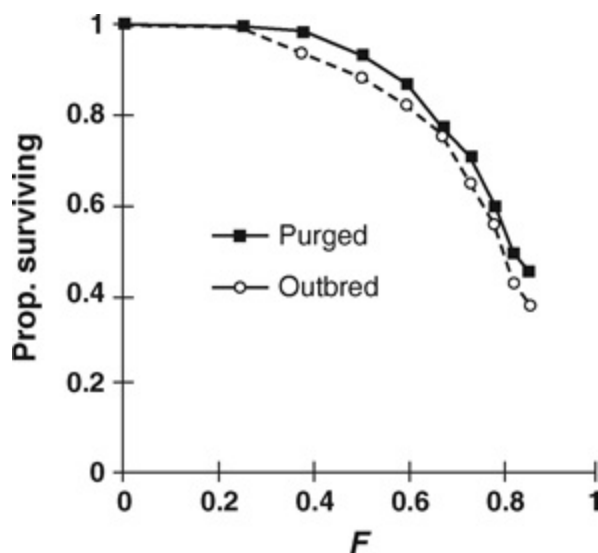
A prior history of small population size is likely to reduce subsequent inbreeding depression, but it is most unlikely to remove it completely

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Some authors have predicted that populations that have been small for many generations, or populations or species with low heterozygosity, will not show inbreeding depression if inbred further (Lande & Schmeske 1985).

However, experimental evidence indicates that purging effects are modest and that small partially inbred populations usually continue to exhibit inbreeding depression when inbred further, even when they have low genetic diversity (Latter *et al.* 1995; Ballou 1997; Boakes *et al.* 2007). For example, a comparison of inbreeding depression in populations derived from an outbred base, with that in populations from crosses between highly inbred populations (purged), yielded a small and non-significant difference between the two treatments (Fig. 13.6). Further, naked mole rats that habitually inbred showed strong inbreeding depression during a coronavirus outbreak (Ross-Gillespie *et al.* 2007).



**Fig. 13.6** Effect of purging on extinctions due to inbreeding. The proportion of populations surviving at different inbreeding coefficients in outbred and purged populations subjected to continuous full-sib mating. The purged populations were formed from four-way crosses among highly inbred populations (20 generations of full-sib mating prior to crossing) from the outbred population (after Frankham *et al.* 2001). *The difference in extinction rates with inbreeding between purged and non-purged populations was small and non-significant.*

The effects of prior inbreeding are not always consistent; effects ranged from reduced inbreeding depression (purging), through no effect to enhanced inbreeding depression in three deer mice populations (Lacy & Ballou 1998). These results were as predicted from the history of the populations: the first

one was large, the second experienced periodic bottlenecks and the third was small historically.

## Detecting and measuring inbreeding depression

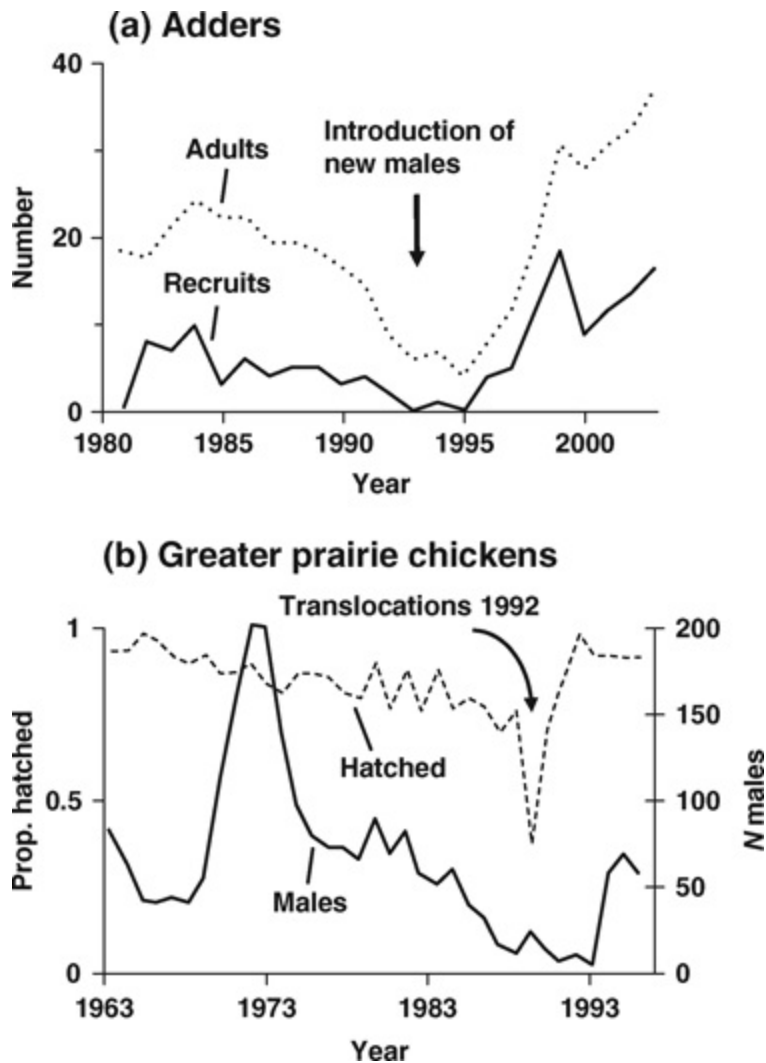
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Contemporary comparison of inbred and outbred individuals (or populations) maintained under the same environmental conditions are required to detect inbreeding depression

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Survival and reproduction are strongly influenced by environmental conditions ([Chapter 5](#)). Consequently, we must compare the fitness of inbred and non-inbred individuals under the same environmental conditions contemporaneously. For example, the deleterious effects of inbreeding on captive mammals were studied by comparing the juvenile survival of inbred and outbred offspring matched for zoo, enclosure in zoo, year of birth and density of population (Ballou & Ralls [1982](#)). Deleterious effects of inbreeding for deer mice in the wild were documented by releasing inbred and outbred offspring of the same age into the wild at the same time and following their subsequent survival and weights (Jimenez *et al.* [1994](#)).

Alternatively, inbreeding depression has been detected by showing that progeny of outcrossing show increased fitness (**heterosis**) compared to contemporary inbred offspring in the same environment. This method has been used for topminnow fish, greater prairie chickens and adders ([Box 13.1](#) and [Fig. 13.8](#) below).



**Fig. 13.8** Genetic rescue due to introduction of immigrants into small partially inbred populations of (a) adders in Sweden (after Madsen *et al.* 2004), and (b) greater prairie chickens in Illinois – translocations began in 1992 (Westemeier *et al.* 1998).

Where pedigrees are not available, genetic markers such as multiple microsatellite loci can be used to infer the degree of inbreeding of individuals, and inbreds and outbreds can then be compared in the same environment. This method has been applied to detect inbreeding depression in harbour seals, Soay sheep, red deer, black grouse and eelgrass (Coltman *et al.* 1999; Slate *et al.* 2000; Höglund *et al.* 2002; Hämmerli & Reusch 2003).

The controversial case of the cheetah illustrates the problems that can arise

in testing for inbreeding depression (see May [1995](#)). As the cheetah has low genetic diversity, it is presumed to be inbred as a consequence of population bottlenecks. However, we cannot determine whether the cheetah, as a species, is suffering from inbreeding depression, as there are no outbred control cheetahs for comparison. However, the current captive cheetah population does exhibit inbreeding depression based upon the fitness of contemporary inbred and outbred individuals (Hedrick [1987](#)).

## Measuring inbreeding depression

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Inbreeding depression is usually measured as the proportionate decline in mean phenotype per unit increase in inbreeding coefficient

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A general measure of inbreeding depression ( $\delta$ ) is the proportionate decline in mean of a phenotypic trait (e.g. fitness) due to inbreeding, as follows:

$$\delta = 1 - \frac{\text{fitness of inbred offspring}}{\text{fitness of outbred offspring}} \quad (13.4)$$

This is simply the measure defined previously ([Equation 13.1](#)), divided by the mean of the outbred population, i.e.  $ID/M_0$ . Example 13.2 illustrates the use of [Equation 13.4](#) to estimate inbreeding depression in Dorcas gazelle. This formula does not specify the level of inbreeding, and this must be defined. The compilation of estimates of inbreeding depression due to sib-mating ( $F = 0.25$ ) in [Table 13.2](#) is presented in this form.

$\delta$  is widely used in plants, where the usual estimate of inbreeding depression is obtained by comparing selfed ( $F = 0.5$ ) and outcrossed progeny.

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### Example 13.2 Inbreeding depression in Dorcas gazelle (Ralls & Ballou 1983)

Juvenile survival of 50 outbred and 42 inbred Dorcas gazelle were 72.0% and 40.5%. The inbreeding depression ( $\delta$ ) for juvenile survival in this species is:

$$\delta = 1 - \frac{\text{fitness of inbred offspring}}{\text{fitness of outbred offspring}} = 1 - \frac{0.405}{0.720} = 0.44$$

The average inbreeding coefficient for the inbred individuals was approximately 0.31.

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## Lethal equivalents

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The usual means for expressing and comparing the extent of inbreeding depression for survival in animals is lethal equivalents

Lethal equivalents are estimated from the slope of the regression of survival on level of inbreeding. The probability of surviving  $S$ , can be expressed as a function of inbreeding  $F$  (Morton *et al.* 1956):

$$S = e^{-A} e^{-BF} = e^{-(A+BF)} \quad (13.5)$$

where  $e^{-A}$  is survival in an outbred population,  $e^{-BF}$  the survival due to inbreeding,  $F$  the inbreeding coefficient and  $B$  the rate at which survival

declines with a change in inbreeding.  $B$  measures the additional genetic damage that would be expressed in a complete homozygote ( $F = 1$ ) and is the number of lethal equivalents per gamete, and  $2B$  the number per individual. A lethal equivalent is defined as a group of detrimental alleles that would cause on average one death if homozygous, e.g. one lethal allele, or two alleles each with 50% probability of causing death, etc. As this definition often causes confusion, a few examples are given. A haploid lethal equivalent of  $B = 1$  means that survival is reduced by 22% ( $1 - e^{-1 \times 0.25}$ ) at  $F = 0.25$  and by 63% at  $F = 1$ , while  $B = 2$  means that survival is reduced by 39% at  $F = 0.25$  and by 86% at  $F = 1$ . Example 13.3 illustrates the computation of the reduction in survival due to 3.14 lethal equivalents as found by Ralls *et al.* (1988) for captive mammals.

---

### Example 13.3 Computing effects of a given level of lethal equivalents and inbreeding coefficient on survival

Ralls *et al.* (1988) reported that the median level of lethal equivalents for juvenile survival in 38 mammal populations was 3.14 diploid lethal equivalents ( $2B$ ). The juvenile survival ( $S$ ) in the progeny of full-sibs compared to the progeny of unrelated individuals is determined using Equation 13.5:

$$\frac{S_{\text{inbred}}}{S_{\text{outbred}}} = \frac{e^{-(A+BF)}}{e^{-A}} = e^{-(BF)}$$

Upon substituting  $B = 3.14/2 = 1.57$  and  $F = 0.25$ , we deduce that relative survival in the progeny of full-sibs is reduced to

$$\frac{S_{\text{inbred}}}{S_{\text{outbred}}} = e^{-(BF)} = e^{-(1.57 \times 0.25)} = 0.67$$

Thus, survival in the progeny of full-sibs is reduced by  $(1 - 0.67) \times 100 = 33\%$ .

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If we take natural logarithms (ln) Equation 13.5 becomes:

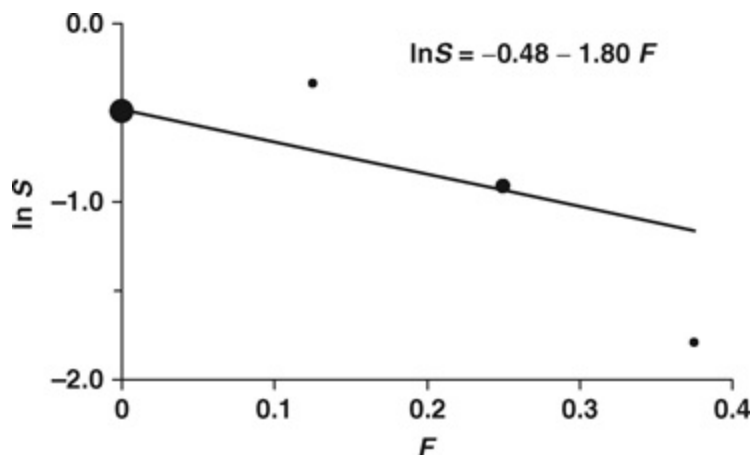
$$\ln S = -A - BF \quad (13.6)$$

Thus, we expect a linear relationship between  $F$  and natural logarithm of survival. To estimate lethal equivalents, data are collected on survival rates of individuals with different levels of  $F$  and weighted linear regression used to estimate  $A$  and  $B$ , as illustrated for the okapi in Table 13.4 and Fig. 13.7. The slope of the regression line ( $B$ ) is  $-1.80$ , indicating that the population contains 1.8 haploid and 3.6 diploid lethal equivalents.

**Table 13.4** *Data on survival levels for offspring with different levels of inbreeding ( $F$ ) in the okapi*

$F$	Lived	Died
0	86 (61%)	55 (39%)
0.125	5 (71%)	2 (29%)
0.25	12 (40%)	18 (60%)
0.375	1 (17%)	5 (83%)

Source: de Bois *et al.* (1990).



**Fig. 13.7** Relationships between survival and inbreeding coefficient in okapi (de Bois *et al.* 1990). The natural logarithm of survival is plotted against the inbreeding coefficient and the linear regression line is inserted (there is a suspicion that the relationship may be curvilinear, but this was not

evaluated due to low numbers).

However, wild populations typically exhibit much higher levels of inbreeding depression than captive populations, averaging ~12 diploid lethal equivalents for total fitness (O'Grady *et al.* 2006). For example, a wild song sparrow population in nature exhibited 12.5 diploid lethal equivalents for total fitness (Keller 1998; Problem 13.6), a wild mouse population exhibited 6.75 diploid lethal equivalents for total fitness (Meagher *et al.* 2000) and a wild wolf population displayed 6.04 lethal equivalents for number of surviving pups per litter during first winter (Liberg *et al.* 2005).



Okapi

## Genetic rescue

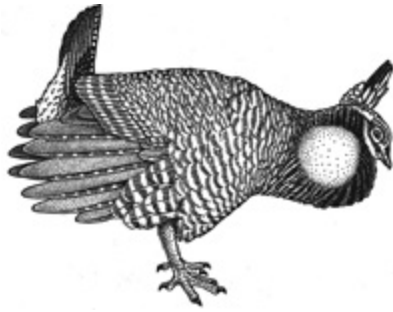
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Inbreeding depression is reversed by outcrossing

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Outcrossing has frequently been used to reverse inbreeding depression, especially in laboratory and agricultural species (Spielman & Frankham 1992; Falconer & Mackay 1996). More recently it has been used to recover

small, inbred populations of several species of wild plants and animals, including bighorn sheep, deer mice, Florida panthers, gray wolves, lions, Mexican wolves, greater prairie chickens ([Fig. 13.8b](#)), Swedish adders ([Fig. 13.8a](#)), desert topminnow fish ([Box 13.1](#)) and several species of plants (Willi *et al.* 2007; [Chapter 2](#)). Such crosses for **genetic rescue** can be made with an outbred population, or to another, independent, inbred population.



Greater prairie chicken

Crossing different populations may result in **outbreeding depression**, a reduction in fitness in hybrids due to genetic differences between the parental populations (see [Chapter 16](#)). However, when the taxonomy is clearly understood, and the populations come from similar habitats, the benefits of crossing nearly always outweigh the risks of outbreeding depression, especially when the populations involved previously experienced gene flow ([Chapter 16](#)).

## Summary

1. Inbreeding results in a decline in reproductive fitness (inbreeding depression), in essentially all well-studied, naturally outbreeding populations of animals and plants.
2. All components of reproductive fitness are subject to inbreeding depression.
3. Inbreeding depression is much greater for total fitness than for its components.
4. The expression of inbreeding depression is typically greater in harsher environments than in benign ones, including wild compared

to captive.

5. Inbreeding depression has a large stochastic element due to different contents of deleterious alleles in different species, families and populations and to the chance element of Mendelian inheritance.
6. Inbreeding depression is predominantly due to homozygosity for partially recessive deleterious alleles, with a modest contribution from loci exhibiting overdominance.
7. The extent of inbreeding depression is  $\Sigma 2pqdF$ . Thus, it depends on the number of loci polymorphic for deleterious alleles, their heterozygosity and directional dominance, and is proportional to the amount of inbreeding.
8. Inbreeding depression is measured by lethal equivalents, or as the proportionate change in mean ( $\delta$ ) for a given level of inbreeding.
9. Deleterious alleles of large effect may be purged (reduced in frequency) from inbred populations, but mildly deleterious alleles are likely to remain and overdominant loci are not subject to purging.
10. Inbreeding depression occurs in selfing species, but is generally of a lesser magnitude than that found in naturally outbreeding species.
11. Outcrossing reverses the effects of inbreeding (genetic rescue).

### **Further reading**

Charlesworth & Charlesworth (1987, 1999) Excellent review of theory and empirical issues relating to inbreeding depression.

Falconer & Mackay (1996) *Introduction to Quantitative Genetics*. Provides a clear treatment of inbreeding depression.

Hedrick & Kalinowski (2000) A review on inbreeding depression and its importance in conservation biology.

Keller & Waller (2002) A fine review of inbreeding depression in wild habitats.

Lynch & Walsh (1998) *Genetics and Analysis of Quantitative Traits*. Chapter 10 has a comprehensive review of theory and experimental evidence on inbreeding depression.

Ralls *et al.* (1988) Highly influential study on the effects of inbreeding

depression for captive wildlife.

## Software

Regression of phenotypic value on inbreeding coefficient can be done with a wide array of statistical software, including Microsoft EXCEL, MINITAB, SAS, SPSS, STATISTICA and VASSARSTATS (see [Chapter 5](#)).

## Problems

- 13.1 Inbreeding depression. What determines the level of inbreeding depression experienced by a population?
- 13.2 Inbreeding depression. What effect does inbreeding have on a haploid organism?
- 13.3 Inbreeding depression. How much inbreeding depression in survival is expected due to selfing for the following three loci, each with allele frequencies of  $0.9 + : 0.1 m$ ?

	$+/+$	$+/m$	$m/m$
(a) Survival % (partial dominant)	90	89	70
(b) Survival % (overdominant)	80	90	70
(c) Survival % (additive)	90	80	70

- 13.4 Inbreeding depression  $\delta$ . The juvenile survival of inbred and outbred offspring in the pygmy hippopotamus were 45% and 75%, respectively (see [Fig. 12.1](#)). What is the inbreeding depression as measured by  $\delta$ ?
- 13.5 Lethal equivalents. Compute the regression equation relating  $\log_e$  juvenile survival in *Parma wallabies* and inbreeding coefficient ( $F$ ), given survival of individuals with inbreeding coefficients  $F$  of 0, 0.625, 0.125, 0.25 and 0.375 are 80%, 68%, 59%, 43% and 31%. How many lethal equivalents are there (a) per haploid genome? (b) per diploid genome?
- 13.6 Lethal equivalents, survival and inbreeding depression. Compute the number of haploid and diploid lethal equivalents for Keller's ([1998](#)) data for song sparrows where he found that 'on Mandarte

Island, an egg with an inbreeding coefficient of  $F = 0.25$  thus experienced on average a total loss of fitness of 79%’.



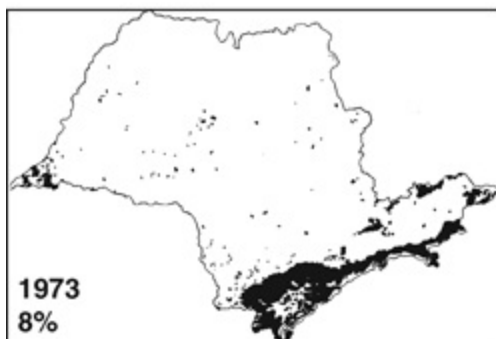
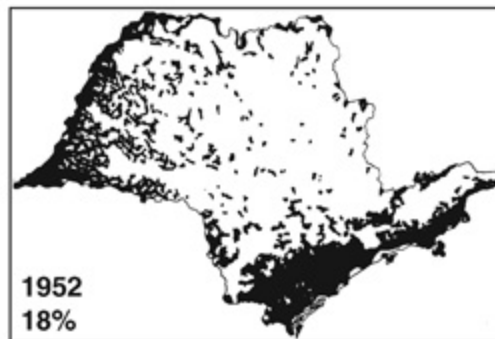
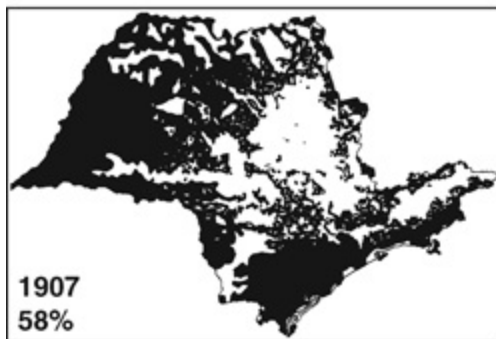
## Chapter 14 Population fragmentation

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The genetic impacts of population fragmentation depend critically upon gene flow among fragments. Fragmentation with cessation of gene flow is highly deleterious in the long term, leading to greater inbreeding, more loss of genetic diversity and elevated extinction risk, when compared to a single population of the same total size

### Terms

Assignment test, Bayesian,  $F_{ST}$ ,  $F$  statistics, landscape genetics, metapopulation, single large or several small (SLOSS), source–sink, spatial autocorrelation, Wahlund effect



Fragmentation of Atlantic forest in São Paulo State, Brazil, from 82% forest cover in 1500 to 3% in the year 2000 (from Oedekoven [1980](#))

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## Habitat fragmentation

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Habitat fragmentation is the conversion of once-continuous habitat into a patchwork with reduction of total habitat area, and isolation of different patches

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Habitat fragmentation includes two processes, a reduction in total habitat area and creation of separate ‘island’ patches from a larger continuous distribution, usually in a matrix of now-inhospitable terrain. These are evident for the Atlantic forest in São Paulo State, Brazil (chapter frontispiece), and parallel examples abound. Human-induced habitat losses and fragmentation are recognised as the primary causes of biodiversity loss (UNEP [2007](#)).

Habitat fragmentation leads to overall reductions in population size for most species, and to reduced migration (gene flow) among patches. The deleterious consequences of reduced population size on genetic diversity, inbreeding and extinction risk have been addressed in [Chapters 2](#) and [11–13](#).

This chapter focuses on the genetic effects of **population fragmentation**, the separation of a population into partially or completely isolated fragments. In particular, we compare the genetic impacts of fragmented populations with a single population of the same total size (**single large or several small** or SLOSS).

## Population fragmentation

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The genetic consequences of population fragmentation depend critically upon gene flow. With restricted gene flow, fragmentation is highly deleterious in the long term

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Inhospitable, cleared habitat among fragments typically inhibits gene flow. Roads, rivers, mountains, etc. may also act as genetic barriers. For example, there is very limited gene flow for bobcats and coyotes across the Ventura Freeway near Los Angeles, USA (Riley *et al.* [2006](#)). The effects of fragmentation on gene flow depend on:

- number of population fragments
- distribution of population sizes in the fragments
- distance between fragments
- geographic distribution or spatial pattern of populations
- dispersal ability of the species
- migration rates among fragments
- ability of immigrants to establish and breed
- environment of the matrix among the fragments and its impact on dispersal
- time since fragmentation, and
- extinction and recolonization rates across fragments.

All of the issues surrounding loss of genetic diversity and inbreeding depression derived from reduced population size come into play when populations are fragmented, and are usually more severe than in a non-fragmented population of the same total size. Fragmentation therefore often results in elevated extinction risks.

The endangered red-cockaded woodpecker in the eastern USA illustrates

many of the features and genetic problems associated with habitat fragmentation for a species with a once continuous distribution. These include loss of genetic diversity, differentiation among populations and isolation by distance (Box 14.1). These effects are also found in plants (Honday & Jacquemyn 2007).

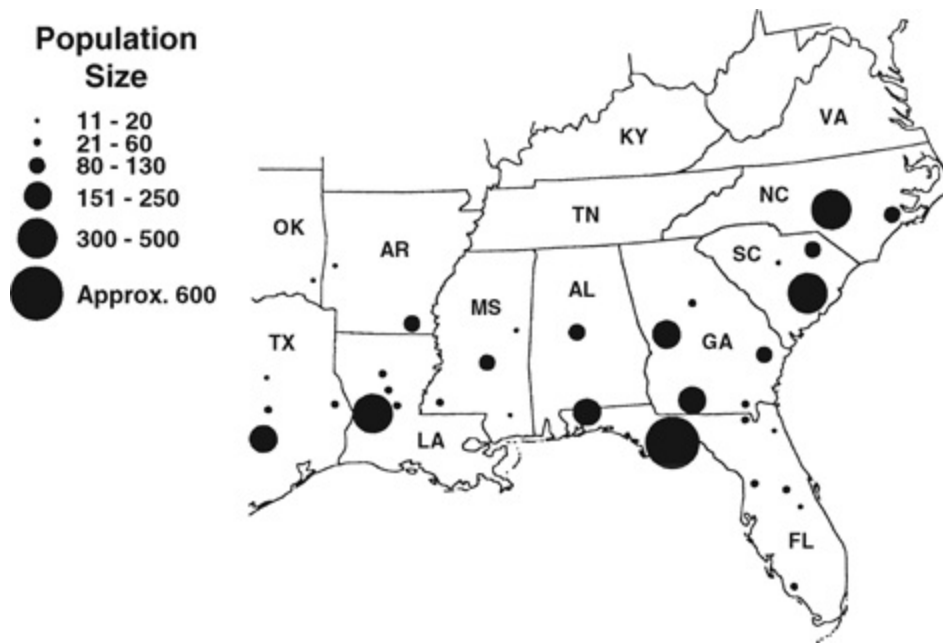
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**Box 14.1 Impact of habitat fragmentation on the endangered red-cockaded woodpecker metapopulation in southeastern USA (Stangel et al. 1992; Kulhavy et al. 1995; Daniels et al. 2000)**

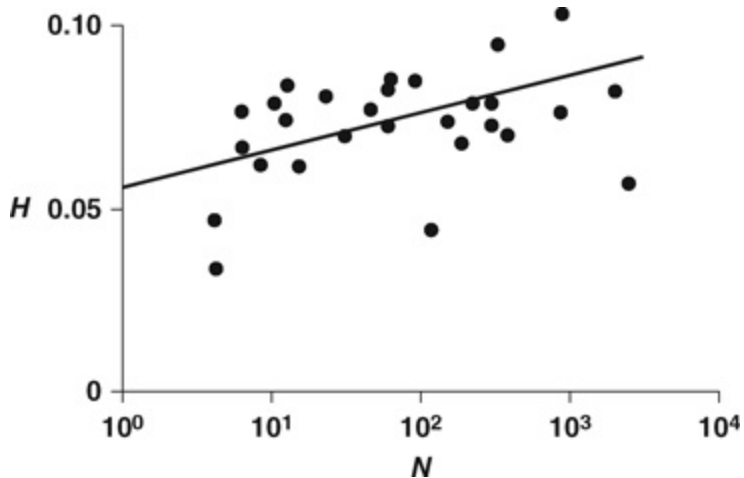
The red-cockaded woodpecker was once common in the mature pine forests of the southeast United States. It declined in numbers, primarily due to habitat loss, and was placed on the US endangered species list in 1970. It now survives in scattered and isolated sites (see map from James 1995). A species recovery plan is being implemented to manage the species.



Red-cockaded woodpeckers

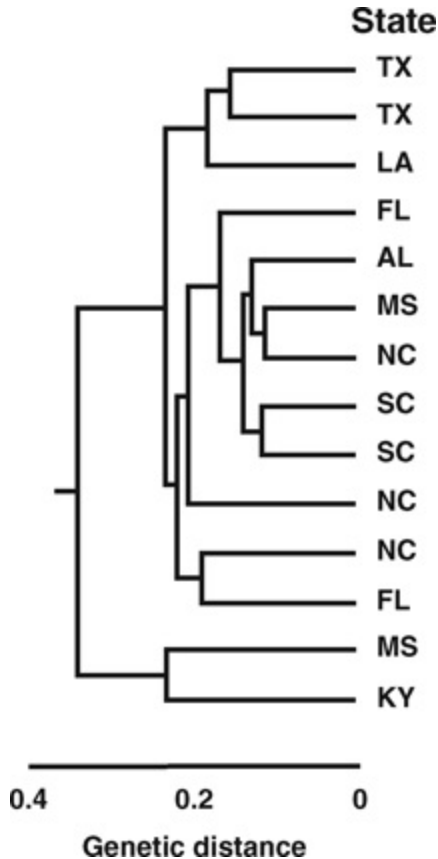


As there is little migration and gene flow among isolated sites, populations have lost genetic diversity and diverged genetically from each other. Smaller populations show lower genetic diversity than larger ones (after Meffe & Carroll [1997](#)).



Moderate divergences in allele frequencies exist among woodpecker populations. Differentiation, measured as  $F_{ST}$  ( $F_{ST} = 0$  indicates no differentiation, and 1.0 equals complete isolation, as described later in this chapter), is 0.14 based on allozyme data, and 0.19 based on RAPD data. There is generally closer genetic similarity among geographically proximate populations – shown in the cluster analysis of genetic distances

among 14 populations in the margin (after Haig & Avise 1996).



Computer simulations indicate that the smallest woodpecker populations are likely to suffer from inbreeding depression in the near future (Daniels *et al.* 2000).

The distribution and density of the woodpeckers is restricted by their requirement for old-growth forest which provides nest holes. Steps have been taken to preserve such forest for the woodpeckers. However, hurricanes damage their habitat and kill birds. In 1989 Hurricane Hugo destroyed 87% of the nesting trees in Francis Marion National Forest in South Carolina, and killed 63% of the birds. This population had recovered by 33% by 1992, due mainly to the installation of artificial nest cavities.

Management of the woodpeckers involves habitat protection, construction of artificial nest holes, reintroductions to re-establish extinct populations, and augmentation of small populations to minimize

inbreeding and loss of genetic diversity. Recovery guidelines specify an effective size of 500 for each major fragment.

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In this chapter we describe alternative population structures, consider the genetic impacts of completely isolated fragments (the most extreme case), discuss the impact of migration and gene flow, present means for measuring genetic divergence and inferring rates of gene flow ( $F$  statistics) and, finally, delineate the genetic impacts of different population structures.

## Population structure

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The impact of population fragmentation depends on the details of the resulting population structure

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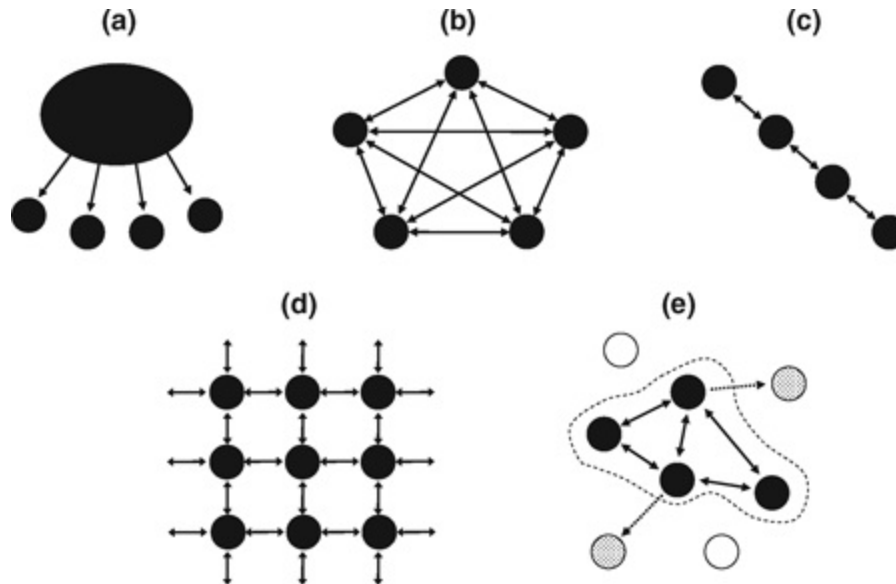
The genetic impacts of population fragmentation may range from insignificant to severe, depending upon the details of the resulting population structures and gene flow among fragments. Several theoretical models of fragmented population structures can be distinguished ([Fig. 14.1](#)):

- totally isolated population fragments with no gene flow ('islands')
- effectively single large – fragments where gene flow is sufficient to result effectively in a single large population
- island structure where migration is equal among equally sized islands
- linear stepping-stone structure, where only neighbouring populations exchange migrants (as in riparian habitat along rivers)
- two-dimensional stepping-stone structure, where only surrounding



populations exchange migrants

- mainland–island or source–sink structures, and
- metapopulations.



**Fig. 14.1** Five different fragmented population structures: (a) a mainland–island (or source–sink) situation, where the ‘mainland’ (source) provides all the input to the island (sink) populations, (b) an island structure, where migration is equal among equal-sized islands, (c) a linear stepping-stone structure, where only neighbouring populations exchange migrants, (d) a two-dimensional stepping-stone structure, where neighbouring populations exchange migrants (all after Hedrick 1983) and (e) a metapopulation–empty circles indicate extinct populations and dotted ones newly founded populations (after Hanski & Gilpin 1997).

The last five cases are illustrated in Fig. 14.1.

**Metapopulations** differ from the other structures in that there are regular extinction and recolonization events, while no extinction is assumed in the simpler forms of the other theoretical structures (Hanski & Gaggiotti 2004). For example, there are about 1600 suitable meadows for the endangered Glanville fritillary butterfly population in Finland, 320–524 being occupied in 1993–1996, with an average of 200 extinctions and 114 colonizations per year. In general, the genetic consequences of a metapopulation structure are

more deleterious than for other population structures (apart from completely isolated fragments).

Extensive population genetic theory has been developed to model genetic processes for different populations structures.

## What is a population?

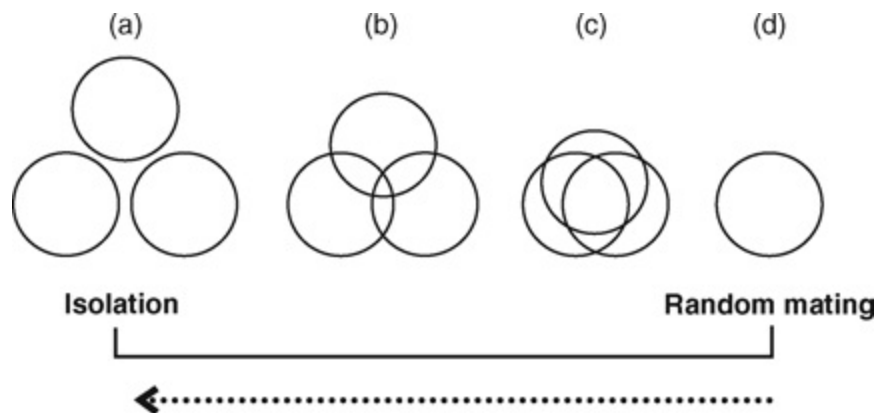
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We define a population as a group of individuals living in sufficiently close proximity that any member of the group can potentially reproduce with any other member

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So far, we have not defined what we mean by a population. There are at least 16 definitions of population, reflecting ecological, evolutionary and statistical paradigms (Waples & Gaggiotti [2006](#)). Since we are concerned with evolutionary issues in conservation genetics, we have followed their evolutionary definition of a population (margin box).

There is a continuum between completely isolated populations and completely connected ones (random mating) depending upon the level of gene flow and divergence ([Fig. 14.2](#)). Reproductive cohesiveness is determined by levels of gene flow. As we shall see below, this depends upon the number of migrants exchanged each generation.

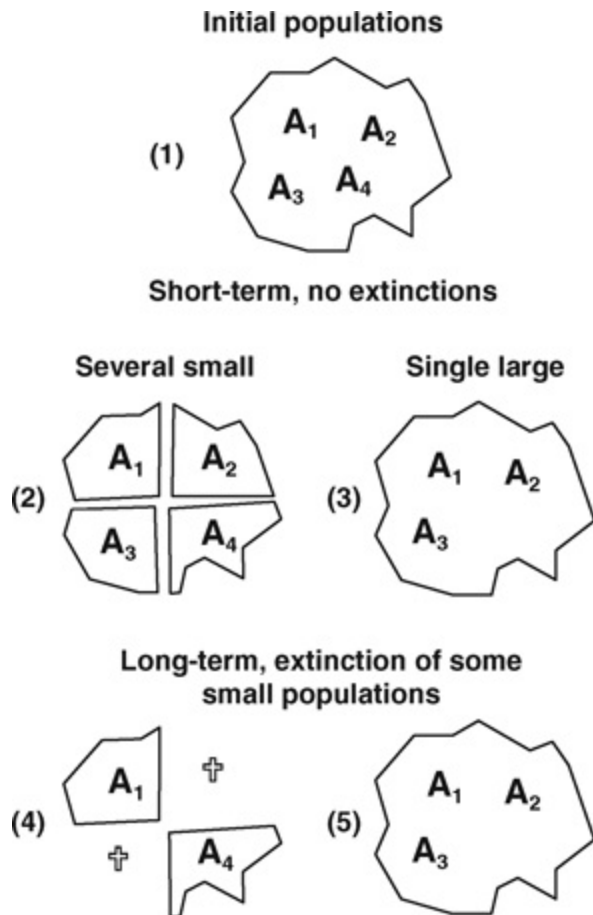


**Fig. 14.2** The continuum of population differentiation (after Waples & Gaggiotti 2006). Each group of circles represents a group of sub-populations with varying degrees of connectivity (geographical overlap and/or migration). (a) Complete independence, (b) modest connectivity, (c) substantial connectivity, and (d) random mating; ‘sub-populations’ are completely congruent.

## Completely isolated population fragments

Fragments that are completely isolated suffer loss of genetic diversity, increased inbreeding and elevated extinction risk, compared to a single large population of the same total size

The short- and long-term genetic consequences of population fragmentation compared to a single large population of the same total size are illustrated in Fig. 14.3. The deleterious effects are particularly evident in the long term. Below, we develop theory to quantify these effects.



**Fig. 14.3** The genetic consequences of a single large population (SL) versus several small (SS) completely isolated population fragments of initially the same total size (SLOSS) over different time frames. (1)  $A_1$ – $A_4$  represent four alleles initially present in the population. In the short term, without extinctions, the several small populations (2) are individually expected to go to fixation more rapidly, but overall to retain greater genetic diversity than the single large population (3). The chances are greater that an allele will be totally lost from the large population, than from all of the small populations combined. However, the SS populations will each be more inbred than the SL population.

In the longer term, when extinctions of small, but not large, populations occur, the sum of the small surviving populations (4) will retain less genetic diversity than the single large population (5).

### Isolated population fragments as ‘islands’

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Isolated population fragments share many of the features of island populations

As we have described earlier, island populations are often inbred, have lower genetic diversity and elevated extinction risks compared to mainland populations ([Chapter 2](#)). For example, island populations of black-footed rock wallabies in Western Australia possess far fewer microsatellite alleles per locus than mainland populations ([Table 14.1](#)). Further, the island populations differ in the alleles they contain, in a more or less random manner. The one population whose fitness has been examined (Barrow Island – BI), exhibits inbreeding depression in female fecundity ([Box 2.3](#)). While these island populations have been isolated for ~1600 generations, loss of genetic diversity can occur over many fewer generations. This is already evident in three species of mammals on islands created, in 1987, by damming of a river in Thailand (Srikwan & Woodruff [2000](#)).

**Table 14.1** Loss of genetic diversity in black-footed rock wallaby populations fragmented on islands by sea level rises 8000–15 000 years ago. Alleles present (+) and absent (–) at four microsatellite loci in populations on the Australian mainland and on six offshore islands (Eldridge *et al.* 1999). *Island populations contain far fewer alleles than mainland populations, but they are usually a sub-set of those found on the mainland. Different island populations often contain different alleles, as expected from genetic drift*

Locus	Allele	Mainland	Islands					
			BI	SI	PI	MI	Wil	Wel
Pa297	102	+	—	—	—	—	—	—
	106	+	—	—	—	—	—	—
	118	+	—	—	—	—	—	—
	120	—	—	—	+	—	—	—
	124	+	—	—	—	+	—	—
	128	+	—	+	—	—	+	+
	130	+	—	—	—	—	—	—
	136	+	+	—	—	—	—	—
Pa385	157	+	—	—	—	—	—	—
	159	+	—	—	+	—	+	+
	161	+	—	+	—	—	—	—
	163	+	—	—	—	+	—	—
	165	+	—	—	—	—	—	—
Pa593	173	—	+	—	—	—	—	—
	105	+	—	—	—	—	+	+
	113	—	+	—	—	—	—	—
	123	+	—	—	—	—	—	—
	125	+	—	—	+	—	—	—
	127	+	—	—	—	—	—	—
	129	+	—	—	—	—	—	—
	131	+	—	+	—	—	—	—
	133	+	—	—	—	—	—	—
	135	+	—	—	—	—	—	—

Locus	Allele	Mainland	Islands					
			BI	SI	PI	MI	Wil	Wel
Me2	137	—	—	—	—	+	—	—
	216	+	—	—	—	—	—	—
	218	+	—	—	—	+	+	—
	220	+	+	—	—	—	—	+
	222	+	—	+	—	—	—	—
	224	+	—	—	—	—	—	—
	230	—	—	—	+	—	—	—



Black-footed rock wallaby

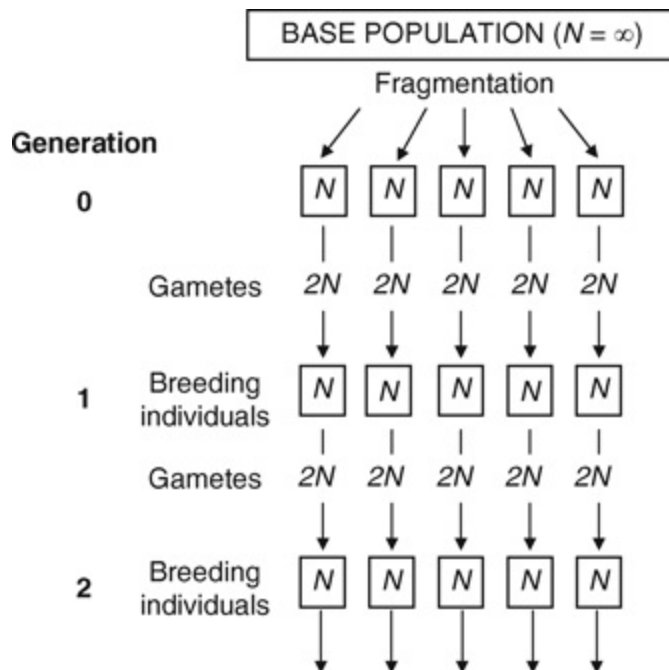
## Consequences of fragmentation in an idealised population

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Deleterious genetic impacts accumulate over time within isolated fragments and are more severe in the smaller fragments

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We begin by considering an idealized fragmented population ([Fig. 14.4](#)) and evaluating the impacts of totally isolated populations (all of equal sizes) on (a) diversity in allele and genotype frequencies among fragmented populations and (b) divergence of these frequencies among replicate populations over generations.



**Fig. 14.4** Model of population fragmentation in an idealized population. The base population is infinite, the populations founded from it are of equal size, and there is no migration among them. Each of the individual fragmented populations is itself an idealized population (after Falconer & Mackay 1996 p.50).

It is useful to consider fragmentation as occurring in two steps:

- (1) fragmentation resulting in an initial genetic sub-division of a population, and
- (2) cumulative diversification, through genetic drift and inbreeding, over time in each of the population fragments.

## Initial generation effect

Differentiation in allele frequencies among fragmented populations is greater for small than for large fragments



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When a population is fragmented by chance, different fragments will have different initial allele frequencies. This diversification is typically measured as variances in allele frequencies ([Box 5.3](#)) and can be predicted from the binomial sampling variance. Consider a single locus with two alleles,  $A_1$  and  $A_2$ , at frequencies of  $p$  and  $q$  in the base population. For each fragment, we sample  $N$  individuals ( $2N$  gene copies) from the base population. The binomial variance in the frequencies of  $A_1$  among the fragments,  $\sigma_p^2$ , will be:

$$\sigma_p^2 = \frac{pq}{2N} \quad (14.1)$$

Thus there is greater differentiation of allele frequencies among small fragments than among large fragments (Example 14.1). Further, variance is greatest when initial allele frequencies are equal (0.5), as  $pq$  is at a maximum with these frequencies.

---

### **Example 14.1 Variance in allele frequencies in different sized population fragments**

Consider a locus with initial allele frequencies of 0.6 and 0.4. In replicate fragments with sizes of 100, the variance of allele frequencies among fragments is ([Equation 14.1](#)):

$$\sigma_p^2 = \frac{pq}{2N} = \frac{0.6 \times 0.4}{(2 \times 100)} = 0.0012$$

Fragments of size 10 have a variance of allele frequency of:

$$\sigma_p^2 = \frac{pq}{2N} = \frac{0.6 \times 0.4}{(2 \times 10)} = 0.012$$

Thus, variance in allele frequencies, after sampling to form fragments, is directly proportional to sample size.

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Heterozygosity in fragmented populations will be lower than in the original continuous population and will vary among fragments.

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The average reduction in heterozygosity due to sampling from the base population is  $1/(2N)$  (Equation 8.2). This initial reduction is minor unless the population fragments are very small (e.g. less than 10). This effect was illustrated in Table 8.2 for population fragments each founded with a single pair of parents.

## Multigeneration effects

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Allele frequencies continue to diversify among fragments by genetic drift over generations until all populations reach fixation

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Diversification in allelic frequencies continues generation after generation until, eventually, all populations are fixed ( $p = 1$ , or 0). By extending Equation 14.1 over multiple generations, the expected variance in allele frequencies among fragments after  $t$  generations is:

$$\sigma_p^2 = p_0 q_0 \left[ 1 - \left( 1 - \frac{1}{2N} \right)^t \right]$$

(14.2)

where  $p_0$  and  $q_0$  are the initial allele frequencies and the fragment size ( $N$ ) is constant over time.

From this equation, we predict that the variance in allele frequencies will

- increase with generations ( $t$ ), and
- increase faster in smaller than in larger populations.

The theoretical distributions of  $p$  expected after different numbers of generations are shown for two initial values of  $p$  in [Fig. 14.5](#). With time, the distributions become flattened as allele frequencies disperse, resulting in an essentially uniform distribution after  $2N$  generations (excluding the fixed populations).

The observed distribution for small fruit fly populations ([Fig. 14.6](#)) is similar to that expected. Dispersion in allele frequencies among populations is also evident in red-cockaded woodpeckers ([Box 14.1](#)) and black-footed rock wallabies ([Table 14.1](#)). The predictions are illustrated numerically in [Example 14.2](#). When the number of generations is so large that all populations have become fixed, the variance is  $p_0q_0$ .

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### **Example 14.2 Increase in variance of allele frequencies with time**

Buri's fruit fly experiment ([Fig. 14.6](#)) began with  $p = q = 0.5$  and each of the populations had 16 parents per generation. The expected variances in allele frequencies after the first generation, computed using [Equation 14.2](#)

is

$$\sigma_p^2 = pq \left[ 1 - \left( 1 - \frac{1}{2N} \right)^t \right] = 0.5 \times 0.5 \left[ 1 - \left( 1 - \frac{1}{(2 \times 16)} \right)^1 \right] = 0.0078$$

After two generations

$$\sigma_p^2 = pq \left[ 1 - \left( 1 - \frac{1}{2N} \right)^t \right] = 0.5 \times 0.5 \left[ 1 - \left( 1 - \frac{1}{(2 \times 16)} \right)^2 \right] = 0.015$$

and after 19 generations

$$\sigma_p^2 = pq \left[ 1 - \left( 1 - \frac{1}{2N} \right)^t \right] = 0.5 \times 0.5 \left[ 1 - \left( 1 - \frac{1}{(2 \times 16)} \right)^{19} \right] = 0.113$$

The variance in allele frequencies increases progressively and reaches its maximum value of  $pq = 0.25$  when all populations have become fixed.

The observed variance in the fruit fly experiment increased more rapidly than predicted, as the effective population size ( $N_e$ ) was less than the 16 ( $N$ ) parents used.

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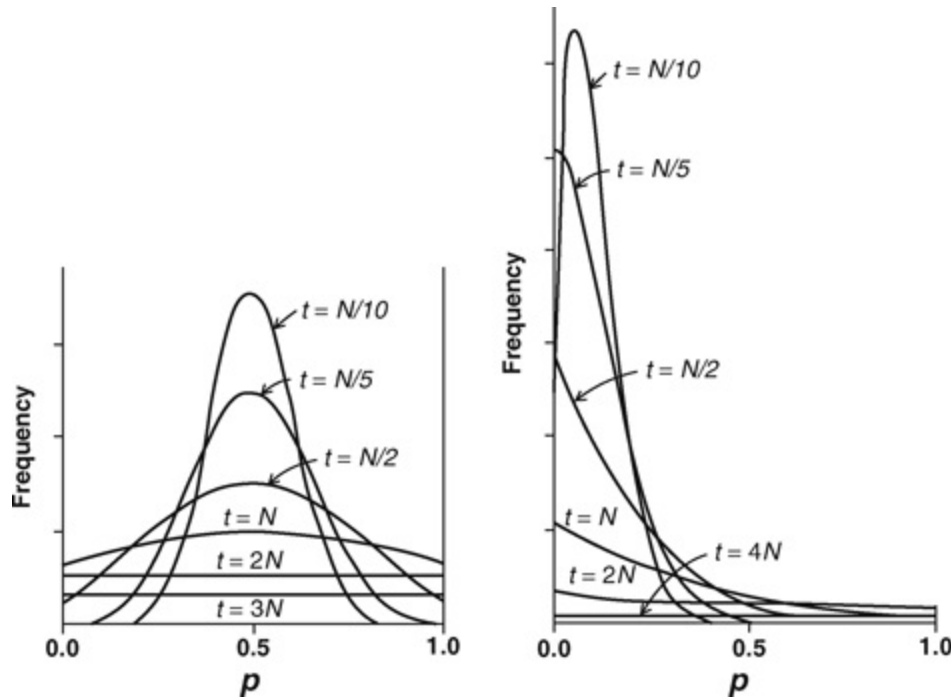
Genetic drift among fragmented populations reduces heterozygosity across all fragments to less than that expected for Hardy–Weinberg equilibrium for the total population.

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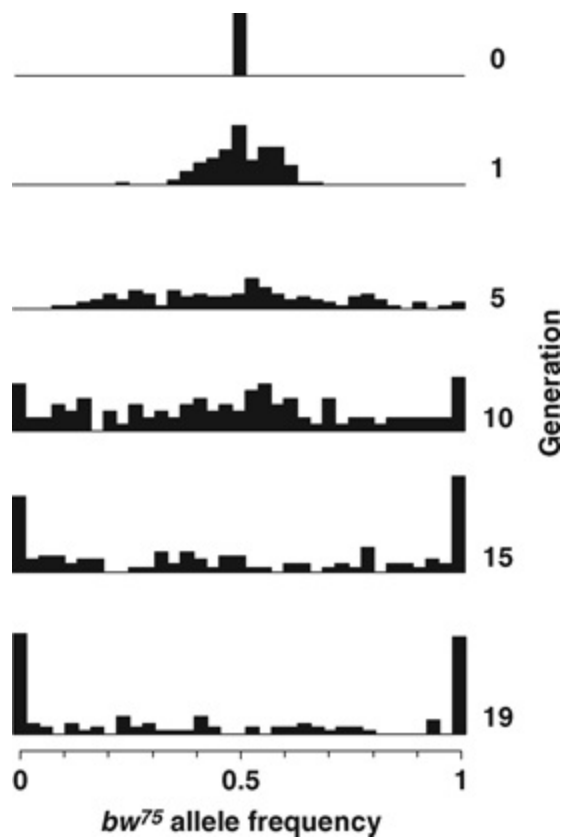
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Population fragmentation results in a deficiency of heterozygotes when compared to Hardy–Weinberg expectations for the entire population ([Table 14.2](#)). This is referred to as the **Wahlund effect** after its discoverer. Consider

a number of fragments, each founded with initial frequencies  $p = q = 0.5$ . Heterozygosity is maximal ( $2pq = 0.5$ ) at the outset. However, as drift occurs in each fragment, frequencies deviate from  $p = q = 0.5$  and  $2pq$  is inevitably reduced (e.g. if two fragments drift to  $p = 0.3, q = 0.7$  and  $p = 0.7, q = 0.3$ , then average heterozygosity is 0.42). If there are many fragments, then the average  $p$  and  $q$  will remain close to 0.5, but the average heterozygosity will be reduced.



**Fig. 14.5** Predicted distributions of allele frequencies among fragmented populations, after different numbers of generations ( $t$ ), expressed in terms of the population size of the fragments ( $N$ ). In the left-hand figure  $p_0 = 0.5$ , and in the right  $p_0 = 0.1$ . Fixed populations are excluded. The horizontal axis is the allele frequency ( $p$ ) in any line. The vertical axis is the probability, scaled to make the area under each curve equal to the proportion of unfixed lines (after Falconer & Mackay 1996).



**Fig. 14.6** Divergence in allele frequencies over time resulting from population fragmentation in fruit flies. The frequency distribution for the  $bw^{75}$  allele is shown over 19 generations in 105 replicate populations maintained with 16 parents per generation. All populations began with initial frequencies of 0.5 (after Buri [1956](#)).

**Box 14.3 Impacts of population fragmentation in Cunningham's skink in Australia (Stow & Sunnucks [2004a, b](#))**



Cunningham's skink

Microsatellite analyses show that all populations of rock-dwelling Cunningham's skink showed strong site and high mate fidelity. However, populations fragmented due to deforestation showed reduced dispersal compared to populations in naturally vegetated areas and an increase in relatedness among individuals. While mating preference for unrelated individuals regulated levels of inbreeding, this constraint resulted in a lower proportion of breeding individuals in deforested versus naturally vegetated habitats.

**Table 14.2 Genotype frequencies in the total population (combination of all population fragments) treated as genetic drift or inbreeding processes ( $p_0$  and  $q_0$  are allele frequencies before fragmentation). Note that  $\sigma_p^2 = Fp_0q_0$**

Genotype	Frequency before fragmentation	Frequencies in the total population after fragmentation	
		Genetic drift	Inbreeding
$A_1A_1$	$p_0^2$	$p_0^2 + \sigma_p^2$	$p_0^2 + Fp_0q_0$
$A_1A_2$	$2p_0q_0$	$2p_0q_0 - 2\sigma_p^2$	$2p_0q_0 (1 - F)$
$A_2A_2$	$q_0^2$	$q_0^2 + \sigma_p^2$	$q_0^2 + Fp_0q_0$

Lower than expected heterozygosity can be used to diagnose populations that are genetically fragmented. For example, the endangered spreading avens plant has a heterozygosity of 0.052, averaged across five populations in the eastern USA, but an expected heterozygosity nearly double this value (0.098) (Hamrick & Godt 1996). Analyses indicate that the deficiency of heterozygotes is due to a combination of population fragmentation and inbreeding within populations.

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Loss of heterozygosity in fragmented populations can be treated as either a drift or an inbreeding process

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Table 14.2 presents genotype frequencies across the totality of all population fragments, treated in terms of both genetic drift and inbreeding. Under drift, the reduction in heterozygosity is equal to twice the variance in allele frequency (Falconer & Mackay 1996). Under inbreeding, heterozygosity is reduced in proportion to  $F$  (Equation 12.1). Example 14.3 illustrates the equivalence of the inbreeding and drift approaches.

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**Example 14.3 Reduced heterozygosity in fragmented populations**

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For a locus with two alleles at frequencies of 0.7 and 0.3, the expected Hardy–Weinberg heterozygosity under random mating  $H_e$  is  $2 \times 0.7 \times 0.3 = 0.42$ . However for a population with an inbreeding coefficient  $F$  of 0.64, this is reduced to

$$H_F = 2 \times 0.7 \times 0.3 \times (1 - 0.64) = 0.15$$



The reduction in heterozygosity of  $(0.42 - 0.15) / 0.42 = 64\%$  is directly proportional to the inbreeding coefficient.

If a group of fragmented populations, all with effective sizes 10 per generation, were isolated from each other for 20 generations ( $F = 0.64$ ), the expected variance in allele frequency would be

$$\sigma_p^2 = 0.7 \times 0.3 \left[ 1 - \left( 1 - \frac{1}{(2 \times 10)} \right)^{20} \right] = 0.135$$

These populations would be expected to have an overall heterozygosity of:

$$H_{\text{frag}} = 2p_0q_0 - 2\sigma_p^2 = 0.42 - 2 \times 0.135 = 0.15$$

Again the heterozygosity is reduced below Hardy–Weinberg random mating expectations.

Consideration of fragmentation as either an inbreeding, or a drift process yields identical answers.

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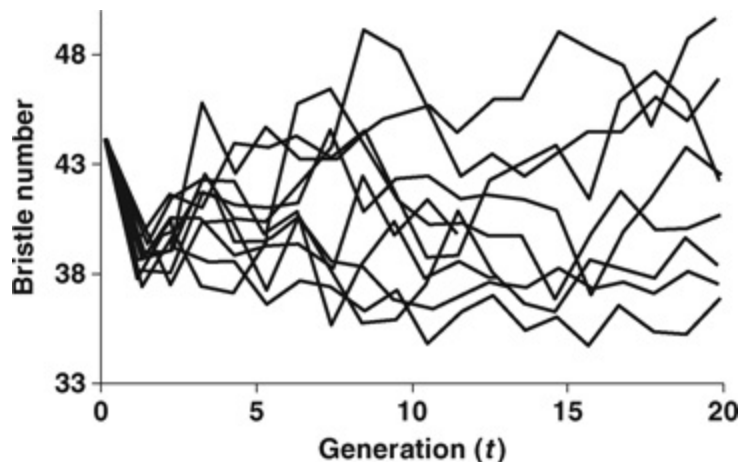
Quantitative characters exhibit diversification in population means due to genetic drift.

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Diversification among fragments in the means for quantitative characters increases with generations in a manner similar to allele frequencies ([Fig. 14.7](#)). Since we cannot distinguish the individual loci and follow their allelic frequencies, changes in additive genetic variances are measured. Additive

genetic variation ( $V_A$ ) among fragments increases and that within populations decreases in parallel with heterozygosity. For additive loci (no dominance) the genetic variance among populations increases to  $2FV_A$ , whilst the genetic variance within populations decreases to  $V_A (1 - F)$  (Falconer & Mackay 1996). With dominance the situation is more complex. However, the genetic variation among populations increases with  $F$ , while the variation within populations may increase initially with  $F$ , before declining to zero at high values of  $F$  (Van Buskirk & Willi 2006).



**Fig. 14.7** Random genetic drift for abdominal bristle number in fruit flies (after Falconer & Mackay 1996, based on Rasmuson). Mean bristle number across generations in 10 populations, all founded from the same base population, and reproduced from a single pair of parents in each generation (two populations became extinct). *Means drift over generations for individual populations and divergence among population means generally increases with generations.*

Each of the many loci affecting a quantitative character undergoes drift independently. Some attain fixation, while others remain polymorphic. The combined effect across all loci in the genome will closely reflect the average effects we have defined above (Table 14.2), i.e. an overall reduction in heterozygosity. However, replicate populations from the same initial base population will have diverse genetic constitutions, as they will vary in the alleles fixed, and in the loci still polymorphic.

## Measuring population fragmentation: F statistics

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The degree of differentiation among fragments can be described by partitioning the overall inbreeding into components within and among populations (F statistics)

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Sewall Wright (Wright 1969) partitioned inbreeding in the total ( $T$ ) population ( $F_{IT}$ ) into:

- inbreeding of individuals relative to their sub-population ( $S$ ) or fragment,  $F_{IS}$ , and
- inbreeding due to differentiation among sub-populations, relative to the total population,  $F_{ST}$ .

$F_{IT}$ ,  $F_{IS}$  and  $F_{ST}$  are referred to as  $F$  statistics (Wright 1969).  $F_{IS}$  is the inbreeding coefficient,  $F$  (Chapter 12), averaged across all individuals from all population fragments.  $F_{ST}$  (referred to as  $G_{ST}$  for multi-allelic loci) is the effect of population subdivision on inbreeding. For a locus with two alleles,  $F_{ST}$  is defined as

$$F_{ST} = \frac{\sigma_p^2}{\bar{p}(1 - \bar{p})} \quad (14.3)$$

where  $\bar{p}$  is the mean frequency of allele and  $\sigma_p^2$  is the variance in frequency of allele  $A_1$  over sub-populations. When all sub-populations have the same allele frequencies, both  $\sigma_p^2$  and  $F_{ST}$  are zero. If the variance is at its binomial maximum  $p(1 - p)$ , then  $F_{ST} = 1$ . Thus,  $F_{ST}$  has a range from 0 (no

differentiation among fragments) to 1 (fixation of different alleles in fragments) for loci with two alleles. Note that  $\sigma_q^2 = \sigma_p^2$ .

$F$  statistics can be calculated using [Equation 12.9](#) which relates heterozygosity and inbreeding. This allows  $F$  statistics to be determined from heterozygosity for genetic markers using the following equations (Nei [1987](#)):

$$F_{IS} = 1 - \frac{H_I}{H_S} \quad (14.4)$$

$$F_{ST} = 1 - \frac{H_S}{H_T} \quad (14.5)$$

$$F_{IT} = 1 - \frac{H_I}{H_T} \quad (14.6)$$

where  $H_I$  is the observed heterozygosity averaged across all population fragments,  $H_S$  is the expected heterozygosity averaged across all population fragments and  $H_T$  is the expected heterozygosity for all populations, treated as a whole (equivalent to  $H_e$ ).

[Table 14.3](#) illustrates the application of  $F$  statistics using hypothetical examples. Case (A) shows two population fragments with identical allele frequencies, but with inbreeding in fragment 2.  $F_{IS}$  is therefore greater than zero. When allelic frequencies are very similar in different fragments, as would be the case when migration is high, or the populations have only recently fragmented, then divergence is low and  $H_S \sim H_T$ , and  $F_{ST} \sim 0$ . In (B) there is no inbreeding within either population fragment. The observed heterozygosity is equal to the expected heterozygosity ( $H_I = H_S$ ), and  $F_{IS} = 0$ . However, the population fragments have different allele frequencies, as will occur with severely restricted gene flow.  $H_T$  exceeds  $H_S$ , and  $F_{ST} > 0$ . In (C) there is both divergence in allele frequencies and inbreeding,  $F_{IS}$  and  $F_{ST}$  are greater than zero and the total inbreeding,  $F_{IT}$ , reflects both effects ([Equation](#)

14.6).

Calculation of  $F$  statistics based on heterozygosities in the endangered Pacific yew in western North America (Example 14.4) reveals inbreeding within populations ( $F_{IS} > 0$ ) and differentiation among populations ( $F_{ST} > 0$ ).

**Table 14.3. Hypothetical example demonstrating calculation of  $F$  statistics from genotype data**



### Example 14.4 Computation of F statistics for the rare Pacific yew

Average observed heterozygosity ( $H_I$ ) for 21 allozyme loci across nine Canadian populations was 0.085, while the average expected heterozygosity for these populations ( $H_S$ ) was 0.166 (El-Kassaby & Yanchuk 1994). Consequently, inbreeding within populations  $F_{IS}$  is

$$F_{IS} = 1 - \frac{H_I}{H_S} = 1 - \frac{0.085}{0.166} = 0.49$$

This high level of inbreeding is not due to selfing as the species is dioecious, but is probably due to offspring establishing close to parents and clumping of relatives from bird and rodent seed caches. The expected heterozygosity for the total nine populations ( $H_T$ ) was 0.18. Thus inbreeding due to population differentiation ( $F_{ST}$ ) is

$$F_{ST} = 1 - \frac{H_S}{H_T} = 1 - \frac{0.166}{0.180} = 0.078$$

This indicates only a modest degree of population differentiation.

The total inbreeding due to both inbreeding within populations and differentiation among them ( $F_{IT}$ ) is

$$F_{IT} = 1 - \frac{H_I}{H_T} = 1 - \frac{0.085}{0.18} = 0.53$$



Pacific yew

$F_{ST}$  can be estimated using allozyme or microsatellite heterozygosity data. A related measure  $R_{ST}$  has also been devised for microsatellite data (Slatkin 1995), but comparative evaluations indicate little difference between this and  $F_{ST}$  for microsatellites (Balloux & Goudet 2002). A more serious issue is that  $F_{ST}$  does not scale 0–1 for markers with more than two alleles, such as microsatellites, and values are not comparable for different microsatellite loci and for allozymes versus microsatellites. To address this problem, Hedrick (2005c) has devised a standardized genetic differentiation measure, based upon scaling  $F_{ST}$  to its maximum value for the given data set.

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$F_{ST}$  increases over generations in fragmented populations at a rate inversely dependent on population size

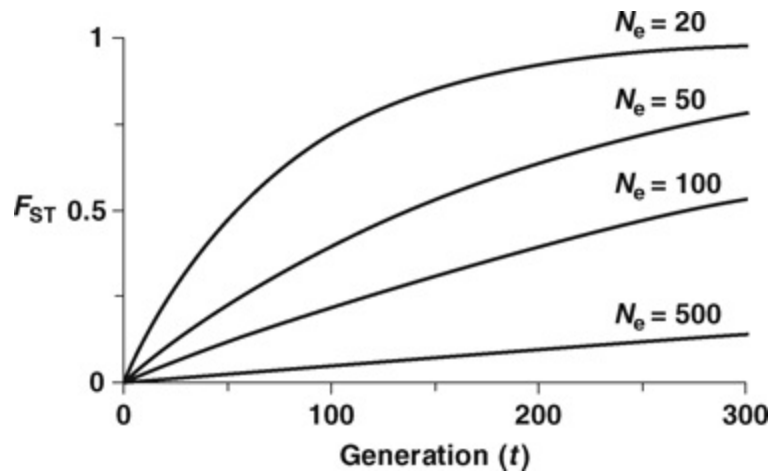
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Figure 14.8 illustrates more rapid increases in  $F_{ST}$  over generations in smaller populations than in larger ones. Typically, an  $F_{ST}$  above  $\sim 0.15$  is



considered to be an indication of significant differentiation among fragments.



**Fig. 14.8** Theoretical increase in  $F_{ST}$  with generations in isolated fragments with different population sizes.

## Gene flow among population fragments

Gene flow reduces the genetic effects of population fragmentation

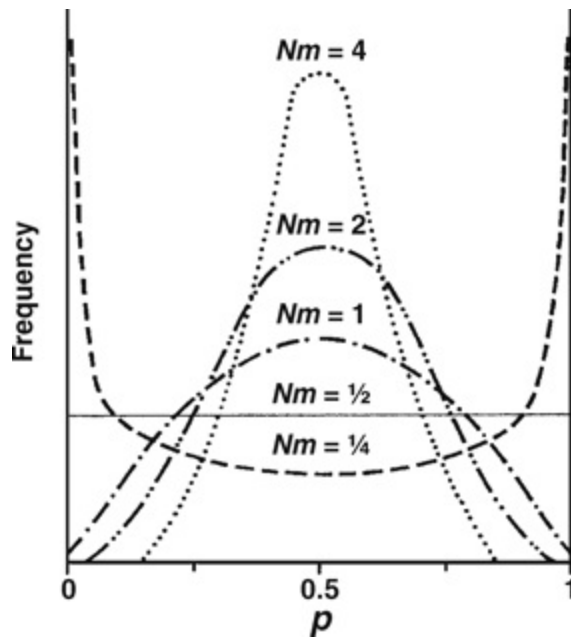
Migration reduces the impact of fragmentation by an extent dependent on the rate of gene flow. With sufficient gene flow a fragmented population will behave as a single large population of the same total size. Many populations exhibit some degree of gene flow among fragments, but less than in the pre-fragmentation continuous population. When is gene flow sufficient to overcome the genetic impacts of fragmentation?

A single migrant per generation is considered sufficient to prevent complete differentiation of idealized populations, irrespective of their size

---

Sewall Wright obtained the surprising result that a single migrant per generation, among idealized populations, was sufficient to prevent complete differentiation (and fixation) irrespective of population size (Wright 1969). This appears paradoxical until it is recognized that one migrant represents proportionally a much higher migration rate in smaller than in larger populations. The higher effective migration rates in smaller populations counteract the greater loss of variation due to drift.

Initially we consider an ‘island model’ where migration rates are equal among identically sized population fragments. Figure 14.9 illustrates the theoretical equilibrium distributions of allele frequencies across population fragments for different migration rates. The migration rate  $m$  is the proportion of genes in a population derived from migrants per generation (Chapter 7), and  $Nm$  is the number of migrants per generation. Populations with migration rates of more than one migrant per generation ( $Nm = 2$  and  $4$ ) exhibit no fixations, while those with less than one migrant per generation ( $Nm = 1/2$  and  $1/4$ ) differentiate to the extent that some populations become fixed for alternative alleles.



**Fig. 14.9** Distribution of allele frequencies in finite populations of size  $N$  with different levels of migration ( $m$ ) among them (after Wright 1940). *Populations with one or more migrants per generation do not differentiate completely (no fixations), while populations with lower migration rates differentiate with a proportion of populations reaching temporary fixation.*

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In real (non-idealized) populations, more than one migrant per generation is required to prevent fixation in population fragments

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More than one migrant per generation is typically required in real populations, as migrants and residents are unlikely to be equally successful in producing offspring, and real populations do not exhibit idealized structures. Numbers of 5, 1–10 and >10 migrants per generation have been proposed (Lacy 1987; Mills & Allendorf 1996; Vucetich & Waite 2000). The

complications arising from non-idealized structures are largely overcome by assessing  $N_e m$  values where a value of 1 has the effect originally envisaged by Wright (Wang [2004a](#)).

## Migration and inbreeding

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Inbreeding is reduced by migration among population fragments

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Inbreeding can be substantially reduced by the introduction of individuals from other fragments ([Chapter 13](#)). This occurs even when the immigrants are themselves inbred, as long as they come from genetically distinct populations.

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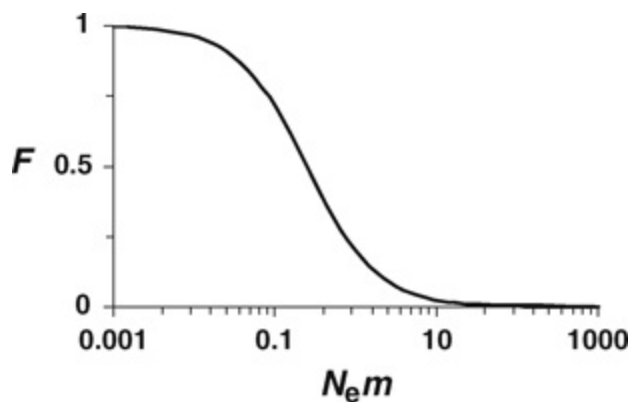
Inbreeding in population fragments depends on the effective population size and the migration rate

---

When population sizes and migration rates are constant, inbreeding and migration reach an equilibrium where the reduction in divergence due to migration balances the increase due to drift. This equilibrium, measured using  $F_{ST}$ , is (Wright [1969](#)):

$$F_{ST} = \frac{1}{4 N_e m + 1} \quad (14.7)$$

This equation applies when  $m$  is small and all population sizes and migration rates among them are the same. [Figure 14.10](#) illustrates the relationship between the number of migrants per generation ( $N_e m$ ) and the inbreeding coefficient. With one migrant per generation,  $F_{ST} = 1 / (4 + 1) = 0.2$ . Conversely, the inbreeding coefficient rises rapidly with less than one migrant per generation, and reaches 1.0 (complete divergence – fixation in populations) when there is no migration.



**Fig. 14.10** Relationship between equilibrium inbreeding coefficient and the number of migrants per generation ( $N_e m$ ) for an island model at equilibrium between drift and migration.

Experimental studies support the expectations above (Bryant *et al.* [1999](#); Newman & Tallmon [2001](#)). For example, populations of the canola plant maintained at sizes of five individuals per generation for five generations, with 0, 1 and 2.5 migrants per generation had inbreeding coefficients of 0.33, 0.08 and 0, respectively, and average seed numbers per plant of 35, 75 and 79.

## Fragmentation in non-idealized populations

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Computer simulations are often used to explore the genetic implications of fragmentation in real populations

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In real populations, the size of the population fragments may differ, there may be different rates of migration among fragments, fragments may have been separated at different times, the individual populations may not have idealized structures and there may be impacts of natural selection. The interactions between these factors can be complex and their effects difficult to predict using theory alone. To predict the genetic consequences of real population fragmentation, computer simulations are often used (McCullough *et al.* 1996; Young & Clarke 2000; Reed 2004).

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For non-idealized populations, the effective population size  $N_e$  is substituted for  $N$  in the appropriate equations

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In real populations, the impacts of fragmentation on genetic divergence, inbreeding and loss of genetic diversity will usually be greater than expected from the census population size, as most populations have  $N_e < N$ , sometimes by orders of magnitude (Chapter 11). We account for this by substituting  $N_e$  for  $N$  in the above equations.

The theory we have just explored assumes selective neutrality of alleles. If alleles are subject to balancing selection, then the rate of diversification will be lower than predicted. If selection differs among patches, such that one allele is favoured in some patches and detrimental in others, then the rate of diversification may be greater than predicted. However, if populations are

small and selection is weak, then alleles will often be effectively neutral ([Chapters 8 and 9](#)).

## Measuring gene flow

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Gene flow can be estimated from the degree of genetic differentiation among populations

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Migration rates are notoriously difficult to measure by direct tracking of individuals, pollen, etc. Further, immigrants may not breed in their new habitat. Gene flow can be inferred from  $F_{ST}$ , as this is related to population size and migration rate by [Equation 14.7](#). For example, by rearranging this equation and inserting the Pacific yew data, we estimate  $Nm = (1 / F_{ST} - 1) / 4 = (1 / 0.078 - 1) / 4 = 2.96$ . On average about three migrants per generation are entering Pacific yew populations. This value reflects historical evolutionary rates of gene flow in equilibrium circumstances, and so may not reflect current gene flow (Steinberg & Jordan [1998](#)). This is an approximation based on the island model, while related expressions have been derived for other models of migration (see Neigel [1996](#)).

While populations do not often strictly adhere to the details of the island model,  $F_{ST}$  is widely used to measure restrictions in gene flow. The exact estimates of migration rates obtained from this equation are not necessarily reliable, but they do indicate the relative rates of gene flow that populations would experience if they adhered to the island population structure.

Several statistical tests are available to detect differences among populations and to estimate rates of gene flow. For reviews of these and

comparisons of their statistical power and characteristics see Latch *et al.* (2006), Ryman *et al.* (2006) and Waples & Gaggiotti (2006).

Cluster analyses based on multilocus microsatellite genotypes in different geographic localities have been used to detect immigrants (assignment tests). These and other means for inferring rates of migration and gene flow are discussed later in the chapter and in [Chapter 21](#).

## Dispersal and gene flow

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Gene flow among fragmented populations is related to dispersal ability

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Since differentiation among populations is dependent on levels of gene flow, we would expect this to be related to the dispersal abilities of species and the degree of isolation among populations. Thus, the degree of genetic differentiation among populations ( $F_{ST}$ ) is expected to be greater for populations:

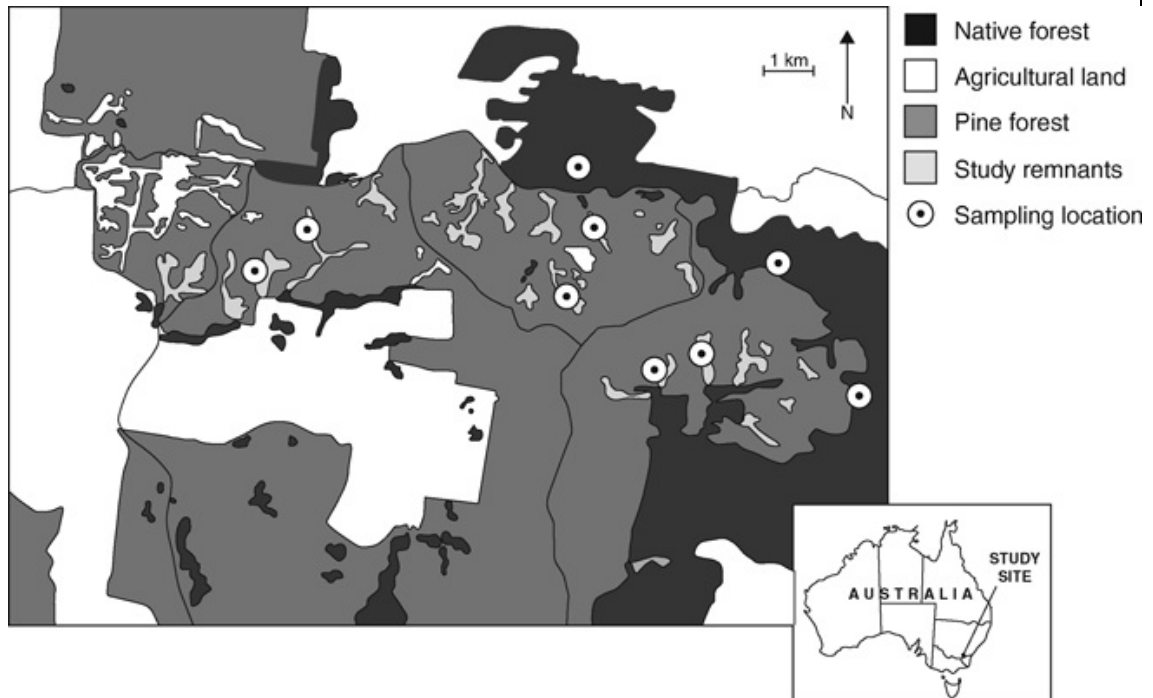
- in species with lower versus higher dispersal rates
- in subdivided vs. continuous habitat
- in distant vs. closer fragments
- in smaller vs. larger population fragments
- in species with longer vs. shorter divergence times (in generations), and
- with adaptive differences vs. those without.

Observations generally confirm these predictions (Hastings & Harrison 1994; Hamrick & Godt 1996). For example, there is a strong negative correlation of  $-0.73$  between  $F_{ST}$  and the dispersal ability of species in a meta-analysis



involving 333 species across 20 animal groups (Bohonak 1999). Mean  $F_{ST}$  values for major groups of organisms are given in Table 14.4. Taxa that can fly, such as birds and insects, have lower  $F_{ST}$  values than those that cannot. Further,  $F_{ST}$  is higher in plants that self (low pollen dispersal) than in outcrossing plants (Table 14.4). Box 14.2 describes the impacts of fragmentation on three species in the Tumut fragmentation study.

**Box 14.2 The Tumut fragmentation study (Lindenmayer & Peakall 2000; Banks et al. 2005a, b; Peakall & Lindenmayer 2006; Taylor et al. 2007)**



A wide-ranging study of the impacts of fragmentation on multiple animal and plant taxa is under way near Tumut in southeastern Australia, under the leadership of David Lindenmayer. A once continuous eucalypt (gum tree) forest was largely replaced by an exotic Monterey pine plantation, following logging between 1949 and 1956. However, 192 small fragments (0.1–125 ha) of eucalypt forest remain within a 50 000-ha habitat matrix of pine that is hostile to most endemic species. Forty-eight unfragmented control sites have been established in nearby

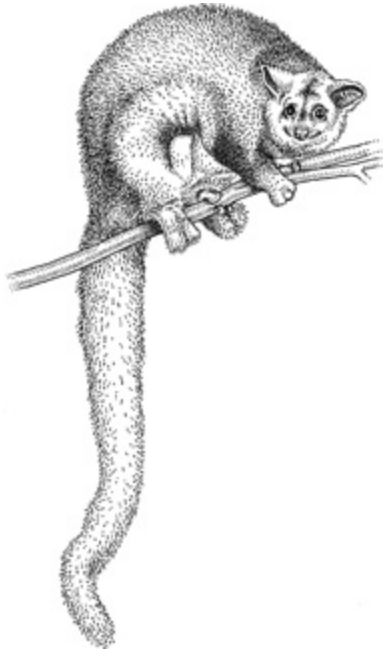
undisturbed eucalypt forest. The genetic impacts of fragmentation have been investigated for Australian bush rats and two marsupials, based mainly upon microsatellite data.

The agile antechinus is a small (20–30 g) marsupial carnivore whose habitat in the Tumut study area has been fragmented for 40–50 generations. Populations do not persist in the pine matrix due to lack of suitable tree hollows for nesting. However, antechinus populations of 3–85 individuals were found in 70% of 23 fragmented patches surveyed. Populations were found in all 48 sites in the undisturbed forest. The genetic diversity of these populations at 15 microsatellite loci and mtDNA d-loop sequence was not significantly lower than that in unfragmented sites, inconsistent with simulated expectations for isolated populations. However, genotypic analyses indicated that large intervening areas of pine matrix (>250 m) reduced dispersal rates among occupied patches. Dispersal through eucalypt forest ‘corridors’ was higher than through the pine matrix. Immigration allows antechinus to persist in neighbouring non-isolated patches, but viable populations do not persist in distant geographically isolated patches due to lack of regular immigration.

Whilst the Australian bush rat is virtually never trapped in the pine matrix, similar levels of genetic diversity were found in six fragments compared to the continuous eucalypt forest ( $H_e = 0.71$  for six microsatellite loci). There were only low levels of genetic differentiation among fragmented populations ( $F_{ST} = 0.044$ ). However, assignment tests detected few immigrants so the current  $F_{ST}$  may not have reached equilibrium levels.

The greater glider is an arboreal, gliding marsupial generally considered conservative in its movements. It is eucalypt-dependent with a diet consisting solely of eucalypt leaves. It showed lower occupancy rates (20%) in patches than in control continuous eucalypt forest sites (38%). Sizes of 11 patch populations ranged from three to 18 individuals. Despite these small sizes and the four to eight generations elapsed since clearing created the patches, genetic diversity for 12 microsatellite loci was relatively similar in patch populations ( $H_e$  0.57–0.81) to that in control sites ( $H_e$  0.70–0.74), and much higher than expected for totally isolated patches. Patch occupancy and genetic analyses indicated at least

some immigration into patches, (presumably by gliding) and assignment tests identified five probable immigrant individuals in patch populations. However, the most geographically isolated and longest isolated patch populations showed restricted immigration.



Greater glider

All genetic studies indicate some immigration through the exotic pine forest matrix, but with dispersal reduced compared to continuous eucalypt forest. The populations may not have reached migration–drift equilibrium and further adverse genetic effects may still occur.

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**Table 14.4   Fixation index ( $F_{ST}$ ) in a range of taxa**

Species	$F_{ST}$	Reference
Mammals (57 species)	0.24	1
Birds (23 species)	0.05	2
Reptiles (22 species)	0.26	1
Amphibians (33 species)	0.32	1
Fish (79 species)	0.14	1
Insects (46 species)	0.10	1
Plants		
Selfing	0.51	3
Mixed selfing and outcrossing		
Animal pollination	0.22	3
Wind pollination	0.10	3
Outbreeding		
Animal pollination	0.20	3
Wind pollination	0.10	3

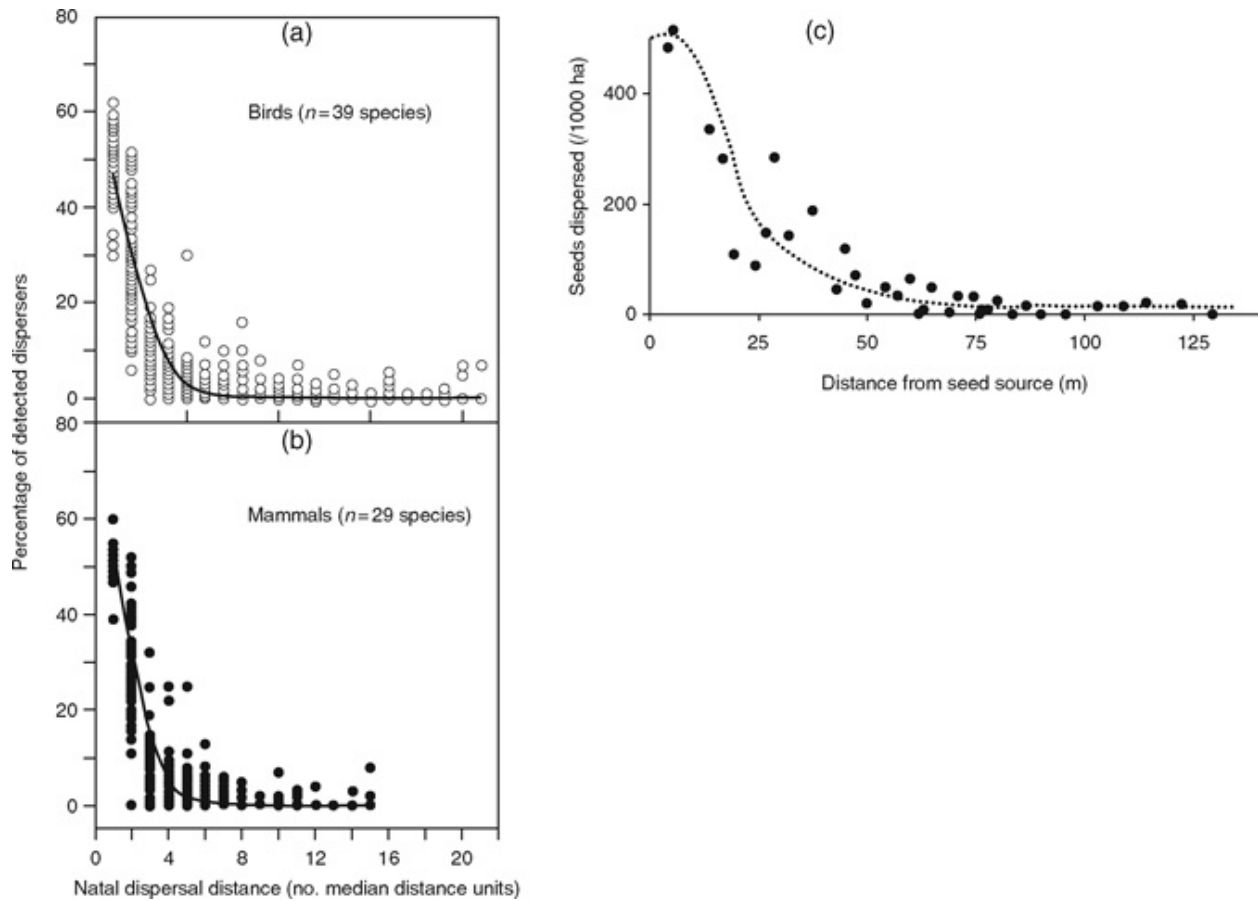
*References:* 1, Ward *et al.* (1992); 2, Evans (1987); 3, Hamrick & Godt (1989).

## Gene flow and distance between fragments

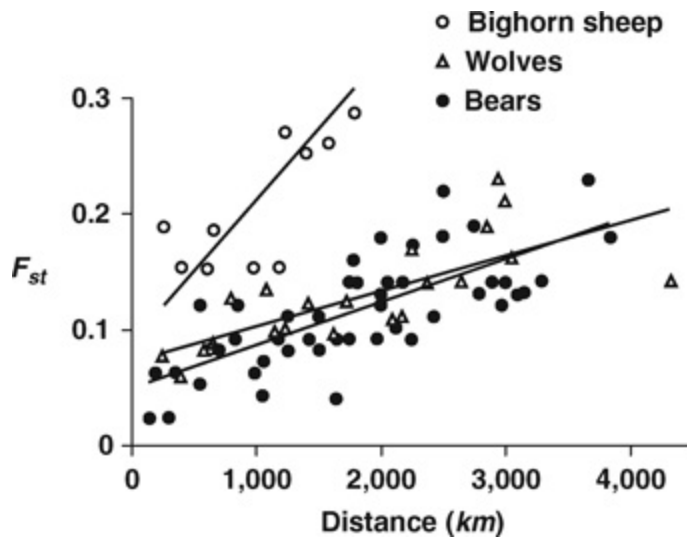
Distant population fragments may exhibit reduced gene flow and isolation by distance

Dispersal rates between populations typically reduce with distance in both animals and plants (Fig. 14.11 previous page). Consequently, genetic differentiation is often related to geographical distance. This is termed **isolation by distance** (Wright 1969). For example, populations of bighorn sheep, gray wolves and bears in North America all show increasing differentiation with geographical distance (Fig. 14.12). Similarly, the red-

cockaded woodpecker (Box 14.1) and the northern spotted owl show relationships between genetic and geographic distances (Haig *et al.* 2001), as do many other species.



**Fig. 14.11** Dispersal distances in (a) birds, (b) mammals (after Sutherland *et al.* 2000) and (c) a eucalypt tree (from Cremer 1966). In (a) and (b) dispersal in different species is expressed as a proportion of the median dispersal for the data set. *Dispersal rates typically decline rapidly with distance.*



**Fig. 14.12** Isolation by distance. Relationship between degree of genetic differentiation ( $F_{ST}$ ) at microsatellite loci and geographic distance among bighorn sheep, brown bear and gray wolf populations in North America (after Forbes & Hogg 1999).  $F_{ST}$  increases with distance in all three species.

However, where dispersal rates are high and distances short, isolation by distance is not expected, as there is sufficient gene flow to prevent differentiation. This has been observed in the plant *Anthyllis vulneraria* in Belgium (Honnay *et al.* 2006).



Brown bear



Gray wolf



Bighorn sheep

## Landscape genetics

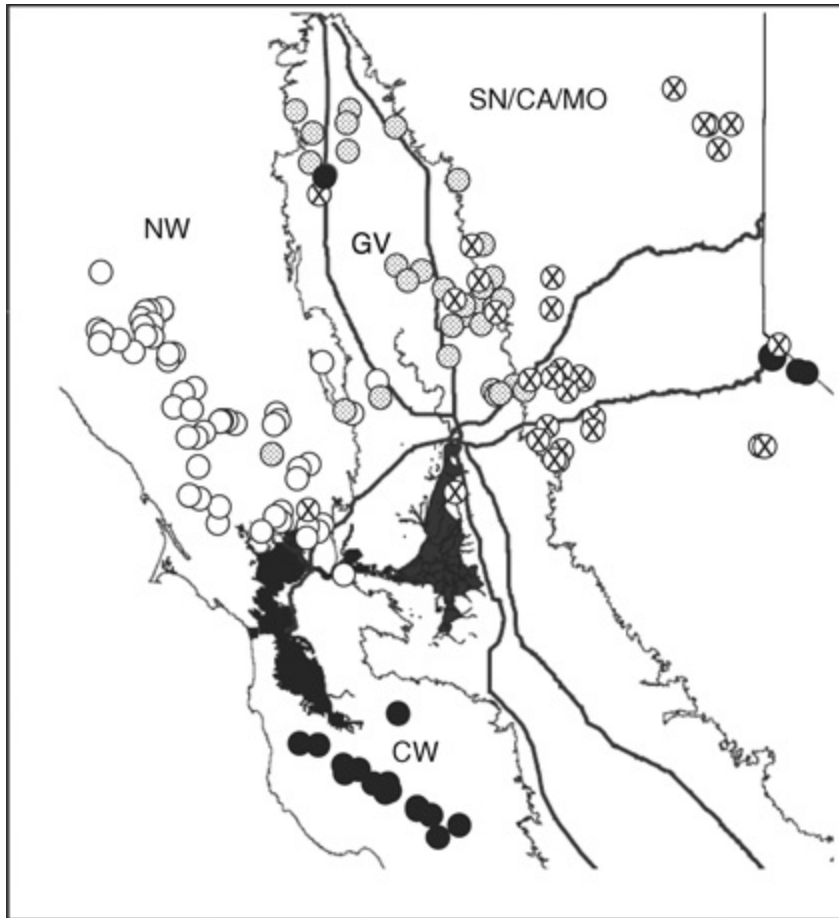
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Analyses of patterns of genetic diversity across the landscape can delineate clines, isolation by distance, genetic boundaries to gene flow, meta-populations and randomness

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We have generally assumed that landscape barriers to gene flow are known. However, the landscape utilization by species and their boundaries are frequently unclear. They can usually be defined by studies that map patterns of genetic diversity onto geography (**landscape genetics**) (Manel *et al.* 2003). Tests for genetic changes across the landscape are typically done using microsatellites, mtDNA sequence data or AFLP. For example, [Fig. 14.13](#) illustrates the clustering of related microsatellite genotypes in different

habitat bioregions across the landscape for coyotes in California, but there are no physical barriers to gene flow.



**Fig. 14.13** Mapping of genetic distinctiveness onto geographical regions for the coyotes in California (after Sacks *et al.* 2004).

Genetic differentiation can also be detected using spatial autocorrelation, a statistical method that tests whether individuals that are adjacent to each other in space are more similar genetically than those further apart. If genetic differentiation increases across a feature of the landscape as determined by spatial autocorrelation, as for example over the long axis of Japan for humans, then we conclude that there is a cline. Boundaries to gene flow may be detected as genetic discontinuities associated with environmental features such as a waterfall in brook char fish, or forest versus grassland habitats in butterflies (Manel *et al.* 2003). Similarly, highways have been shown to impede gene flow in desert bighorn sheep in southern California (Epps *et al.*



2006). Assignment tests in black-tailed prairie dogs identified a metapopulation. Conversely, a random pattern of genetic diversity in the lily *Ornithogalum montanum* was revealed by spatial autocorrelation and by Mantel tests between genetic and geographic distance (Manel *et al.* 2003).

So far, we have considered the consequences of population fragmentations and gene flow on genetic diversity. However, these processes also have major impacts on reproductive fitness via inbreeding, as we describe below.

## Impacts of different population structures on reproductive fitness

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The overall consequences of different population structures on reproductive fitness will depend primarily on the inbreeding coefficient in each fragment

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It is important to define the impacts of different population structures and gene flow rates on reproductive fitness, as this will in turn affect extinction probabilities. Consequences of different population structures, compared to a single large unfragmented population (SL), are as follows.

- In the island and stepping-stone models, inbreeding and fitness will depend critically upon the gene flow and upon the variation in population sizes on different islands (Nunney 1999). When there is no gene flow, inbreeding will depend upon the effective population sizes of the individual populations, and will be greater than for SL. Conversely, when there is ample gene flow among populations, inbreeding will depend upon the effective size of the total population, and be similar to SL.

- In source–sink (or mainland–island) structures, the effective population size will depend on  $N_e$  in the mainland (source) populations, rather than that for the total populations. Thus, inbreeding and loss of fitness are likely to be much higher with this structure than for SL.
- Metapopulations typically have effective sizes that are markedly less than the number of breeding adults, due to cycles of extinction and recolonization (Pannell & Charlesworth 1999; Wang & Caballero 1999; Whitlock 2004). Their inbreeding will typically be greater than in other fragmented and non-fragmented structures and their fitness the lowest.

Deleterious fitness effects of isolation and inbreeding have been demonstrated in wild fragmented populations, along with genetic rescue due to gene flow and beneficial effects of dispersal on extinction risk (Chapter 2). Lowered fitness occurs in fragmented frog populations compared to those with continuous populations (Johannson *et al.* 2007), while fragmentation reduces breeding rates in fragmented populations of an Australian lizard (Box 14.3). Large fitness advantages of gene flow were found in water fleas with a metapopulation structure (Ebert *et al.* 2002). Higher dispersal has been shown to be associated with less loss of genetic diversity and lower population extinction rates in a comparison of two gecko species (Hoehn *et al.* 2007).

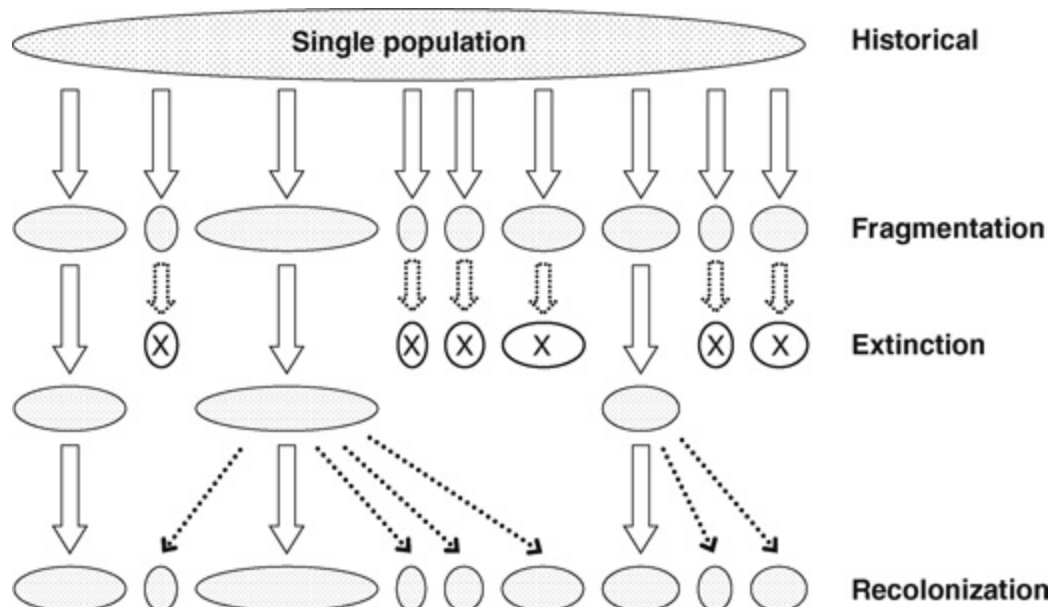
Since metapopulations have features not already considered, further consideration of them follows.

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Extinctions and recolonizations in metapopulations generally lead to more deleterious genetic impacts than for similar-sized fragmented populations with similar gene flow

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Figure 14.14 illustrates extinctions of fragments, and bottlenecks during recolonization, in a metapopulation. Extinctions typically reduce the overall effective sizes to less than that of an equal sized single large population. Bottlenecks during recolonization will subsequently reduce  $N_e$  still further.



**Fig. 14.14** Cycles of extinction and recolonization in a metapopulation, leading to reductions in the effective size of a species. The dotted lines indicate bottlenecks during recolonizations.

If there are frequent extinctions and recolonizations, mainly from a few large fragments, the structure approaches that of a source–sink, and less genetic diversity is retained than in a single large population of the same total size (Gilpin 1991). Conversely, a metapopulation with sufficient migration and low rates of local extinctions approaches the characteristics of a single large population. In general, the higher the rates of extinction and recolonization in a metapopulation, the more severe the genetic impacts on fitness and genetic diversity.

In Chapter 17, we address means for alleviating the deleterious impacts of fragmentation using procedures such as corridors or translocations.

## Summary

1. Population fragmentation usually results in deleterious genetic consequences in the long term, compared to a similar-sized unfragmented population.
2. The genetic effects of fragmentation for the same total population size depend critically upon gene flow. This in turn depends on the number of fragments, population structure and distance among fragments, and upon the characteristics of the species.
3. Inbreeding and reduced fitness in population fragments are more severe with increased fragmentation and lower gene flow, and increase with generations.
4. Fragmented populations diverge in allele frequencies and heterozygosities.
5. Fragmented populations with restricted gene flow share many of the features of island populations, including elevated extinction risk.
6.  $F$  statistics are frequently used to measure differentiation among populations and to infer historic levels of gene flow.
7. Metapopulation structures, with extinctions and recolonizations of population fragments, are often highly deleterious.
8. Analyses of patterns of genetic diversity across the landscape can delineate clines, isolation by distance, genetic boundaries to gene flow, metapopulations and random patterns.

## Further reading

Crooks & Sanjayan (2006) *Connectivity Conservation*. Collection of chapters on fragmentation and population connectivity.

Hanski & Gaggiotti (eds.) (2004) *Ecology, Genetics and Evolution of Metapopulations*. Collection of relevant papers on metapopulations.

Hedrick (2005a) *Genetics of Populations*. [Chapters 9](#) provides a clear treatment of genetic issues relating to population fragmentation.

Manel *et al.* (2003) Excellent review of landscape genetics.

Quammen (1996) *The Song of the Dodo*. An interesting book on island

biogeography and extinction written for a general audience.

Rousset (2004) *Genetic Structure and Selection in Subdivided Populations*. Book on genetics of fragmented populations.

Waples & Gaggiotti (2006) Review on delineating population boundaries.

Young & Clarke (eds.) (2000) *Genetics, Demography and the Viability of Fragmented Populations*. Collection of studies on fragmented populations of animals and plants.

## Software

ARLEQUIN: Calculates  $F_{ST}$  using AMOVA framework (Schneider *et al.* 2000). <http://cmpg.unibe.ch/software/arlequin3/>

BAPS: Bayesian clustering software to estimate the number of populations (Corander & Marttinen 2006). <http://web.abo.fi/fak/mnf//mate/jc/software/baps.html>

EASYPOP: Simulation software for investigating the effects of mutation, population size, migration and population fragmentation (Balloux 2001). [www.unil.ch/dee/page36926\\_fr.html](http://www.unil.ch/dee/page36926_fr.html)

FSTAT: Calculates  $F$  and  $R$  statistics and does partial Mantel test (Goudet 2002). <http://www2.unil.ch/popgen/softwares/fstat.htm>

GenAlEx: Computes basic population genetic statistics, including  $F$  statistics and Mantel tests (Peakall & Smouse 2006). [www.anu.edu.au/BoZo/GenAlEx/](http://www.anu.edu.au/BoZo/GenAlEx/)

GENECLASS2: Free software for genetic assignment and first-generation migrant detection (Piry *et al.* 2004). [www.montpellier.inra.fr/URLB/GeneClass2/Setup.htm](http://www.montpellier.inra.fr/URLB/GeneClass2/Setup.htm)

GENEPOP: Computes  $F$  statistics, isolation by distance and Mantel tests (Rousset 2008). <http://kimura.univ-montp2.fr/~rousset/Genepop.htm>

MIGRATE: Estimates effective population sizes and past migration rates between  $n$  population (Beerli 2006). <http://popgen.scs.fsu.edu/Migrate-n.html>

PARTITION: Package for identifying population subdivision and assigning individuals to populations (Dawson & Belkhir 2001). [www.genetix.univ-montp2.fr/partition/partition.htm](http://www.genetix.univ-montp2.fr/partition/partition.htm)

STRUCTURE 2.2: Uses Bayesian clustering to identify population units (Falush *et al.* 2007). <http://pritch.bsd.uchicago.edu/structure.html>

## Problems

- 14.1 Fragmentation. What are the genetic impacts of population fragmentation?
- 14.2 Variance in allele frequencies. Calculate the variance in allele frequencies for populations with allele frequencies 0.1, 0.2, 0.2, 0.3, 0.3, 0.3, 0.4, 0.4, 0.5 (see Box 5.3 for variances).
- 14.3 Variance in allele frequencies. For many population fragments all derived from an initial population with two alleles at frequencies of 0.3 and 0.7, and maintained as isolated populations with effective sizes of 50, what is the expected variance in allele frequencies (a) after 20 generations, (b) after 100 generations?
- 14.4 Determining migration rates. Calculate  $Nm$  among spotted owl subspecies, given that  $F_{ST}$  is 0.2.
- 14.5 Population structure. Calculate  $H_I$ ,  $H_S$  and  $H_T$  for each of the three situations in Table 14.3.
- 14.6 Population differentiation and  $F$  statistics. Calculate  $F_{IS}$ ,  $F_{ST}$  and  $F_{IT}$  for the spreading avens, an endangered plant endemic to mountaintops in the eastern USA (Hamrick & Godt 1996). Explain what each  $F$  statistic means. Interpret the population structure.

	Observed	Expected
Population	heterozygosity	heterozygosity
PMT	0.056	0.091
RMT	0.050	0.086
GMT	0.049	0.066
CTP	0.054	0.064
CGG	0.050	0.061
Population means	0.052 ( $H_i$ )	0.074 ( $H_s$ )
Species		0.098 ( $H_T$ )

**14.7** Population differentiation and  $F$  statistics. Three populations of the threatened swamp pink plant in the eastern USA showed the following allozyme heterozygosities (Hamrick & Godt 1996). Calculate  $F_{ST}$ .

	Observed	Expected
Population	heterozygosity	heterozygosity
Appalachian	0.038	0.061
Virginia	0.028	0.045
New Jersey	0.021	0.033
Species		0.053

**14.8** Inbreeding and population differentiation. If populations are maintained either as a single population of effective size 50, or two isolated populations of effective size 25 for 30 generations, what are their inbreeding coefficients?

## Chapter 15 Genetically viable populations

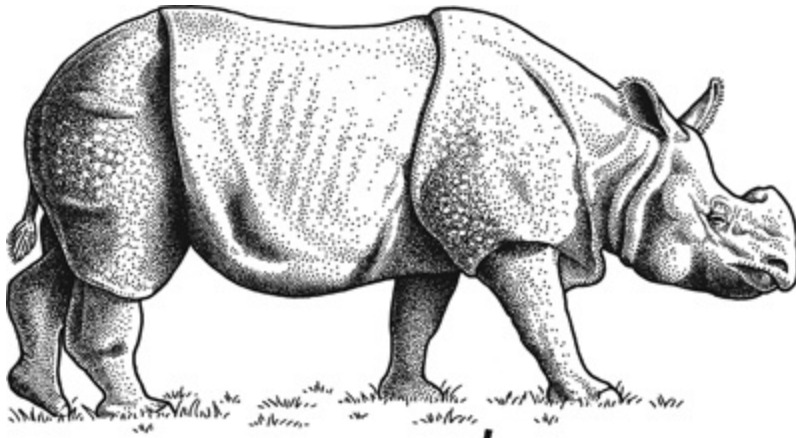
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It is important to define the minimum population size needed to retain genetic 'health', as resources for threatened species are limited. To avoid inbreeding depression and retain fitness in the short-term  $N_e \gg 50$  is required. To permanently retain evolutionary potential,  $N_e$  of 500–5000 is required. Current population sizes of threatened species are typically too small to avoid genetic deterioration

### Terms

Minimum viable population size (MVP), mutational meltdown





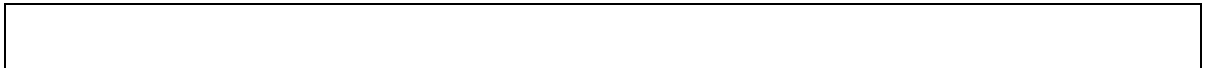
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Endangered Indian one-horned rhinoceros

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**Shortage of space for threatened species**

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There is a severe shortage of space for threatened species, both in wild habitats and in captivity

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The financial and physical resources required to conserve threatened species are enormous, and current resources are grossly inadequate for the task. Habitat loss reduces living space for threatened species. Providing reserves, such as national parks, is costly, and often conflicts with human demands for increased land use. Captive breeding programs are a partial solution, but there is also a chronic shortage of facilities for this strategy. About 4000–6000 threatened vertebrate species require captive breeding, the number having doubled recently due to the amphibian crisis (Anonymous 2006). However, space exists for only about 800 species, although some additional space for amphibians will become available (Tudge 1995). Pragmatic decisions must be made in allocating resources. Retention of too few individuals will lead to the deleterious genetic effects we have discussed, while allocating too much to one species will be at the expense of others.

Consequently, there is an urgent need to define the minimum population size required for species to be viable in the long term. This issue has been discussed under the title of **minimum viable population size** (MVP), yet the population sizes are not necessarily minimum, nor viable. Rather, we are considering the minimum size required to maintain a population that suffers no reduction in reproductive fitness or evolutionary potential over thousands of years. This does not signify that populations of lesser size have no future, only that their reproductive fitness and evolutionary potential are likely to be compromised, with a consequent increased risk of extinction. As Soulé (1987) noted ‘there are no hopeless cases, only people without hope and expensive cases’.

For a particular population or species, this question reduces to:

- is the population size large enough to avoid loss of reproductive fitness?
- does the species have sufficient genetic diversity to evolve in response to environmental change?
- is the population large enough to avoid accumulating deleterious mutations?

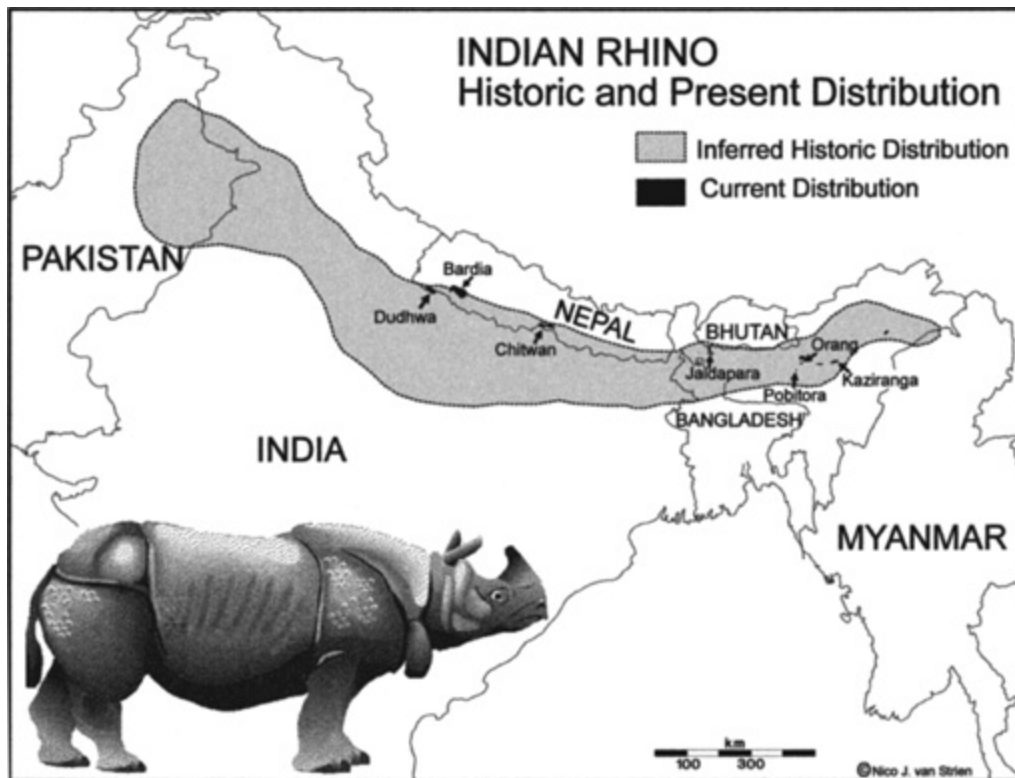
The first two questions are illustrated for the endangered Indian rhinoceros and the northern elephant seal in [Box 15.1](#).

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### **Box 15.1 Is the species genetically viable in the medium to long term?**

#### **Is the rhinoceros population size large enough?**

The Indian one-horned rhinoceros once numbered many hundreds of thousands. With habitat reduction and fragmentation, and poaching for horns, the numbers have been reduced to about 2600 individuals in eight geographically separated areas (International Rhino Fund, pers. comm.). This species has normal levels of allozyme genetic diversity (Dinerstein & McCracken 1990). For the entire species, are there sufficient individuals to avoid extinction due to inbreeding and compromised ability to undergo adaptive evolutionary change? Regrettably, the arguments presented in this chapter lead us to anticipate that the one-horned rhinoceros, and many others, will undergo slow genetic deterioration in the long term.



Area	Population size
Areas with large populations	
Kaziranga (India, Assam)	1825
Chitwan (Nepal)	372
Areas with smaller populations	
Pobitora (India, Assam)	81
Dudhwa/Bardia (Nepal/India)	58
Jaldapara (India, W. Bengal)	180
Orang (India, Assam)	68
Goruma (India, W. Bengal)	27
Manas (India, Assam)	4
Total (includes two other small populations)	2619

### Does the northern elephant seal have enough genetic diversity?

The northern elephant seal underwent a population size bottleneck of about 20–30 individuals, but has since recovered to over 175 000 individuals and is no longer listed as endangered. However, it displays no allozyme genetic diversity (Bonnell & Selander 1974; Hoelzel 1999) and only two mtDNA haplotypes (compared to 24 in related southern

elephant seals). Many other threatened species lack genetic diversity (Chapter 3). Are these species doomed to extinction? Below we will see that such species are likely to have compromised ability to evolve in response to environmental change and thus increased extinction risk. However, they are not predicted to become extinct in the near future, unless they experience an unexpected catastrophe such as a new disease.

## How large?

Various predictions of population sizes required to achieve the goals defined above are given in Table 15.1. We consider the basis for each of these below.

**Table 15.1 How large must populations be to retain genetic ‘health’? Various estimates of the required effective population size ( $N_e$ ) are given, along with the times to recover normal levels of genetic diversity following complete loss of diversity**

Goal	$N_e$	Regeneration time (generations)	Reference
Avoid inbreeding depression	50		1, 2
Retain evolutionary potential	500	$10^2$ – $10^3$	1, 3
	5000		4
	570–1250		5
Retain single-locus genetic diversity	$10^5$ – $10^6$	$10^5$ – $10^7$	3
Avoid accumulating deleterious mutations	1000		4
	100		6
	12		7

*References:* 1, Franklin (1980); 2, Soulé (1980); 3, Lande & Barrowclough (1987); 4, Lande (1995); 5, Franklin & Frankham (1998); 6, Lynch *et al.* (1995a); 7, Charlesworth *et al.* (1993).

## Retaining reproductive fitness

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No finite population is immune to eventual inbreeding depression

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Franklin (1980) and Soulé (1980) both suggested that an effective population size of 50 was sufficient to avoid inbreeding depression, in the short term, based on the experience of animal breeders. An effective size of 50 corresponds to an increase in inbreeding coefficient of 1% per generation. The context of their predictions was that over a period of ~5–10 generations, there would be little detectable inbreeding depression when  $N_e$  was 50. However, inbreeding increases at a rate of  $1/(2N_e)$  per generation, so that all finite closed populations eventually become inbred. Further, as inbreeding depression is linearly related to the inbreeding coefficient (Chapter 13), there is actually no threshold below which inbreeding is not deleterious. Based upon the median number of lethal equivalents found in captive mammals (3.14: Ralls *et al.* 1988;), we would expect about 2% inbreeding depression when  $F = 0.005$ , 4% when  $F = 0.01$ , and 15% when  $F = 0.05$  for juvenile survival alone.

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Populations with effective sizes of 50 in fruit flies and 90 in houseflies show inbreeding depression

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Inbreeding depression has been described in fruit fly populations maintained at  $N_e \sim 50$  for 210 generations (Latter *et al.* 1995) and  $N_e$  of 50, or less, for 50 generations (Woodworth *et al.* 2002). In houseflies inbreeding depression was evident in populations with  $N_e \sim 90$  after only five generations (Bryant *et al.* 1999; Reed & Bryant 2000). For plants, Reed (2005) estimated that a median population size of 2500 was required for long-term maintenance of 95% of initial fitness.

We do not know precisely how large populations must be to avoid meaningful inbreeding depression for fitness over the long term, but the required size is clearly much greater than an effective size of 50. Disturbingly, sizes for threatened and near threatened species in captivity average 113 individuals ( $N_e \sim 33$ ) (ISIS 2007) and about one half of all captive populations of threatened mammals have  $N < 50$  (Magin *et al.* 1994), corresponding to  $N_e < 15$  (Mace 1986), and are likely to suffer inbreeding depression relatively soon.

In practice, wild populations that were listed as endangered in 1985–1991 numbered 100–1000 individuals (Wilcove *et al.* 1993). These correspond to  $N_e \sim 10$ –100, as  $N_e/N$  ratios are typically  $\sim 0.1$  (Chapter 11). Similarly, the IUCN scheme for categorization of extinction risk lists 50, 250 and 1000 adults ( $N_e \sim 5$ , 25 and 100) as cutoffs for the critically endangered, endangered and vulnerable categories (IUCN 2007). Many of these populations have effective sizes of 50 or less and are at risk of extinction from inbreeding depression (without considering other factors) unless their sizes are substantially increased.

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Times to extinction in generations due to inbreeding approximate the effective size of the populations

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Estimated times to extinction for different sized housefly populations approximated the effective size in generations, e.g. 480 generations for  $N_e = 500$  (Reed & Bryant 2000). Extinction risks in rapidly inbred populations of mice and fruit flies increased markedly at  $F = 0.5$  and beyond (Figs. 2.1 and 13.5), and  $F$  values for the housefly populations at extinction lay within a similar range (0.38–0.66).

## Retaining evolutionary potential

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Effective population sizes of 500–5000 have been suggested as necessary to maintain evolutionary potential

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There are a range of estimates of population sizes required to retain evolutionary potential, but there is general agreement that it is an  $N_e$  of at least 500 (Table 15.1). Since the debate about this issue has major implications for the genetic management of threatened species, we consider the estimations in some detail.

Franklin (1980) argued that additive genetic variation, rather than allelic diversity, determined evolutionary potential, and this is directly related to heterozygosity (Equation 5.5). His estimate was based on the balance between loss of quantitative genetic variation and its replenishment by mutation, and was obtained as follows:



$$\Delta V_A = V_m - \frac{V_A}{2N_e} \quad (15.1)$$

where  $\Delta V_A$  is the change in additive genetic variation over one generation,  $V_m$  the gain in genetic variation per generation due to mutation, and  $V_A/(2N_e)$  the loss of additive genetic variation per generation due to drift. At equilibrium,  $\Delta V_A = 0$ , so

$$N_e = \frac{V_A}{2V_m} \quad (15.2)$$

Thus, the required effective population size depends upon the original additive genetic variation and the rate at which it is regenerated by mutation. Empirical data on  $V_A$  and  $V_m$  are required to obtain the required value of  $N_e$ . Estimates of  $V_m$  are obtained from accumulation of new genetic variation in initially homozygous populations and consequently are reported as multiples of the environmental variance ( $V_E$ ; [Chapter 5](#)). Franklin (1980) noted  $V_m \sim 10^{-3} V_E$  for bristle characters in fruit flies (one of the few estimates of  $V_m$  then available). Upon substituting this value into [Equation 15.2](#), he estimated the required  $N_e$  as

$$N_e = \frac{V_A}{(2 \times 10^{-3} \times V_E)} = 500 \frac{V_A}{V_E} \quad (15.3)$$

A value of  $V_A/V_E$  to solve this can be obtained from the heritability, as follows. Since the heritability

$$h^2 = \frac{V_A}{V_P} \sim \frac{V_A}{V_A + V_E}$$

and

$$\frac{V_A}{V_E} \sim \frac{h^2}{1 - h^2}$$

consequently,

$$N_e = \frac{500 h^2}{1 - h^2} \quad (15.4)$$

Franklin (1980) assumed a heritability of 50%, a reasonable estimate of the heritability for peripheral characters (Table 5.3). Consequently, Franklin predicted that an effective size of 500 was required to retain additive genetic variation and long-term evolutionary potential.

Lande & Barrowclough (1987) reached a similar conclusion, based on a model involving an equilibrium between stabilizing selection, drift and mutation. However, Lande (1995) later revised his estimate, arguing that only about 10% of newly generated mutations are useful for future genetic change because most mutations are deleterious. Lande adjusted for the deleterious mutations by using  $V_m = 10^{-4} V_E$  (from data of Lopez & Lopez-Fanjul 1993). Upon substituting this value into Equation 15.2, and using the same heritability as above, he estimated  $N_e$  as 5000 to retain evolutionary potential.

Reservations have been expressed about this high estimate (Franklin & Frankham 1998). First, many estimates of  $V_m = 10^{-3} V_E$  have been derived from long-term experiments where many unconditionally deleterious mutations will have been eliminated. Second, by introducing the issue of deleterious mutations, Lande was considering fitness, rather than peripheral characters. For these, heritabilities are typically 10–20% or less (see Tables 5.2 and 5.3). Using a heritability of 10%, and  $V_m = 10^{-4} V_E$ , we obtain  $N_e \sim 560$ , and for a heritability of 20%  $N_e = 1250$  (Franklin & Frankham 1998). Third, mutations that are deleterious in the current environment may be favourable under altered future conditions. For example, genetic adaptation to captivity appears to arise predominantly from rare alleles that are deleterious in the wild (Frankham 2008). Since evolutionary potential is concerned with the capacity to adapt to environmental change, we need to preserve genetic diversity that is beneficial, deleterious and neutral, in the current

environment.

The calculations above are based on models that ignore natural selection, or do not consider it adequately. Reproductive fitness is the central character for evolutionary potential, but there is currently no theory allowing us to predict the equilibrium additive genetic variation under a model of mutation, drift and directional natural selection operating upon it. Thus, the above estimates of the required  $N_e$  are imprecise, as there are uncertainties about mutational variances for reproductive fitness and about the proportion of mutations that are beneficial, deleterious and conditional in their effects (Keightley & Lynch 2003).

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Wild populations in nature require adult census sizes about 10 times larger than the  $N_e$  values estimated above, i.e. several thousand to tens of thousands

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Since comprehensive estimates of  $N_e/N$  ratios are about 0.1 (Chapter 11), census sizes in wild populations must be about one order of magnitude higher than the  $N_e$  values we have calculated, i.e. 5000–50 000. This sets a lower limit for the minimum size to maintain long-term viability (Soulé 1987), and is within the range of values reached from consideration of other threats (Chapter 22).

## How large are threatened populations?

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Population sizes of endangered species are usually smaller than those required to meet genetic objectives

Actual census population sizes for a variety of endangered species are given in [Table 15.2](#). Most of these have population sizes of fewer than 500 and, presumably, effective population sizes much lower than this. Ellstrand & Elam (1993) reported that 53% of the occurrences of 743 sensitive plant taxa in California contained fewer than 100 individuals.

**Table 15.2 Population sizes in the wild (*N*) and category of endangerment for a variety of threatened taxa. The categories are based primarily on the IUCN system and account for more than population sizes ([Chapter 1](#))**

Species	Location	Category <sup>a</sup>	<i>N</i> <sup>b</sup>	Reference
<b>Mammals</b>				
Asiatic lion	India	Cr. E.	250 mature <sup>c</sup>	I
Eastern barred bandicoot	Australia (mainland)	Cr. E	<1000	I
Ethiopian wolf	Ethiopia	E	442	I
Darwin's fox	Chile	Cr. E	<250 mature	I
Florida panther	USA	Cr. E	30–50 adults	I
Giant panda	China	E	~1600	I

Species	Location	Category <sup>a</sup>	N <sup>b</sup>	Reference
Golden lion tamarin	Brazil	E	>1000 (+ 490 <sup>b</sup> )	1
Humpback whale	Oceans	V	6000	2
Javan rhinoceros	Indonesia	Cr. E	60	3
Northern hairy-nosed wombat	Australia	Cr. E	113	4
Northern Atlantic right whale	E. coast N. America	E	300–350	1
<b>Birds</b>				
Bali starling	Indonesia	Cr. E	25	5
Lord Howe Island woodhen	Australia	E	~160	6
Pink pigeon	Mauritius	E	250	1
Puerto Rican parrot	Puerto Rico	Cr. E	~40	5
Seychelles warbler	Seychelles	V	~1500	7
Red-cockaded woodpecker	USA	V	<10 000 mature	1
Whooping crane	N. America	E	231 (+ 137 <sup>d</sup> )	8
<b>Reptiles</b>				
Aruba Island rattlesnake	Caribbean	Cr. E	350	9
Komodo dragon	Indonesia	V	<3000	10
<b>Invertebrates</b>				
Palo Verdes blue butterfly	California	E (USA)	200	11
<b>Plants</b>				
Apalachicola rosemary	Florida	E	555 <sup>e</sup>	12
<i>Bidens amplexans</i>	Hawaii	V	<1000	1
Catalina mahogany	California	Cr. E	6 <sup>e</sup>	1, 13
Corrigon grevillea	Australia	E	27	14
Mauna Kea silversword	Hawaii	Cr. E	<50 mature	1
Texas snowbell	Texas	E	39 <sup>e</sup>	12
Wollemi pine	Australia	Cr. E	~40 adults	1, 15

<sup>a</sup> Cr. E, critically endangered; E, endangered; V, vulnerable.

<sup>b</sup> Numbers reflect those at the time of the study, and are not necessarily current.

<sup>c</sup> Mature individuals are presumed capable of reproduction under the IUCN

definition.

**d** Captive population.

**e** Prior to captive breeding and reintroduction program.

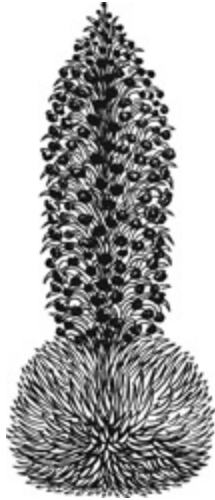
*References:* 1, IUCN (2007); 2, McIntosh (1999); 3, Pain (1998); 4, Banks *et al.* (2003); 5, Seal (1991); 6, NSW NPWS (2002); 7, Komdeur *et al.* (1998); 8, Jones *et al.* (2002); 9, Seal (1992); 10, Ciofi & Bruford (1998); 11, Nelson (1995); 12, Falk *et al.* (1996); 13, Rieseberg & Swenson (1996); 14, Rossetto *et al.* (1995); 15, NSW NPWS (1998).



Eastern barred bandicoot



Puerto Rican parrot



Mauna Kea silversword

## What happens to species with $N_e < 500$ ?

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Species with effective sizes of less than 500 are not doomed to extinction, but will become increasingly vulnerable over time, with increased extinction risk

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Species with effective sizes insufficient for long-term maintenance of genetic diversity are not doomed to immediate extinction. On average they will suffer depletion of genetic diversity, slow inbreeding, reduced fitness and reduced ability to evolve in response to novel environmental threats. They will require increasing human intervention to ensure their survival. This may take the form of provision of more benign environments (isolating them from competitors, avoiding introduction of diseases and improving their environment), or managing them to increase reproduction and survival (e.g. supplementary feeding, as in California condors).

## Reduced long-term evolutionary potential in endangered

## species

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Endangered species have substantially compromised ability to evolve in response to environmental change, as they have low  $N_e$ , and often have reduced reproduction rates and low genetic variation

Response ( $R$ ) to several generations ( $t$ ) of directional selection is predicted as:

$$\sum R = Sh^2 \sum \left(1 - \frac{1}{2N_e}\right)^{t-1} \quad (15.5)$$

where  $S$  = selection differential and  $h^2$  = heritability ([Chapter 5](#)). The sigma term on the right-hand side reflects erosion of genetic diversity each generation due to genetic drift. The total response to selection in the long term, derived from genetic variation in the initial population, is predicted to be approximately  $2N_e Sh^2$  (Robertson [1960](#)).

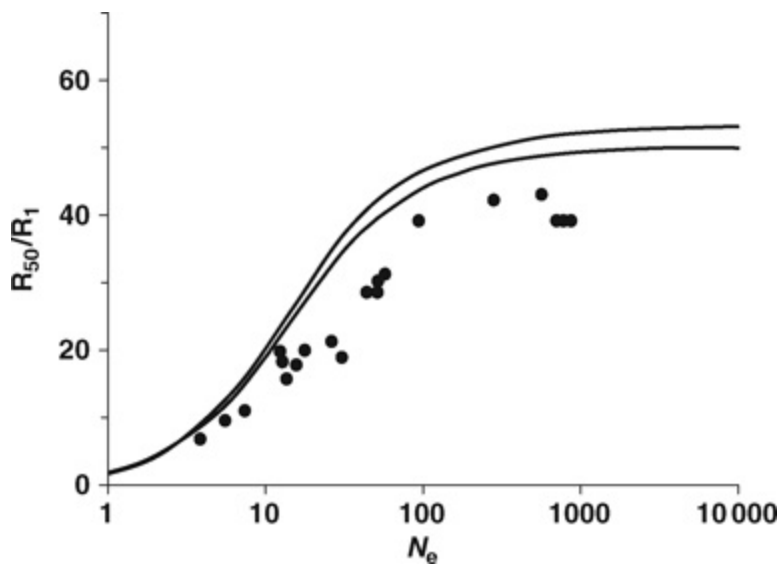
Threatened species will have reduced ability to evolve due to low  $N_e$ . Further, many will have reductions due to lowered  $h^2$ , as threatened species, on average, have 35% lower genetic diversity than taxonomically related non-threatened taxa (Spielman *et al.* [2004a](#)).

For evolutionary change due to new mutations, the asymptotic rate of response per generation after mutation and drift reach equilibrium is predicted to be (Hill [1982](#))



$$R_{\text{mutation}} = \frac{2N_e S V_m}{V_p} \quad (15.6)$$

where  $V_p$  is the phenotypic variance. This provides an approximate prediction of response (Frankham 1983; Mackay *et al.* 1994). Importantly, both prediction equations show that evolutionary potential is an increasing function of  $N_e$ . In practice, selection response increases with population size, but is proportional to  $\log N_e$  rather than  $N_e$  (Fig. 15.1).



**Fig. 15.1** Observed relationship between long-term response to directional selection and effective population size (Weber 2004). Cumulative response at generation 50, divided by the response in the first generation ( $R_{50}/R_1$ ) for selection on a diversity of characters in mice, fruit flies, red flour beetles and maize is plotted against  $\log N_e$  for the populations. The two asymptotic curves represent 50 generation predictions based on Equation 15.5 (lower curve), and with mutation added using Equation 15.6 (higher curve). *Long-term selection response increases with population size.*

Evolutionary change also depends upon reproductive fitness, through the selection differential ( $S$ ) in Equations 15.5 and 15.6. Many threatened species probably have reduced reproductive fitnesses due to inbreeding depression, when compared to taxonomically related, non-threatened species (Spielman

*et al.* [2004a](#)).

Example 15.1 illustrates the potential impacts of the above factors on evolutionary potential in a small island population of gray wolves. They may have  $\sim\frac{1}{2}$  the ability to evolve as the large mainland population.

There are no comparative data on the ability of threatened and non-threatened species to evolve, but anecdotal evidence indicates that it is lower in threatened than in non-threatened taxa. For example, pest insects with large populations have evolved resistance to a wide range of insecticides (McKenzie & Batterham [1994](#)). Conversely, many endangered species, especially those on islands, have succumbed to environmental change in the form of introduced predators, pests, parasites and disease.

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### **Example 15.1 Impact of population size on evolutionary potential (Wayne et al. [1991](#))**

We illustrate the possible impact of restricted population size on evolutionary potential, using the Isle Royale gray wolf. We assume that a single pair founded this population on Isle Royale in Lake Superior, as indicated by genetic data. Consequently, heterozygosity of the island population will be  $\sim(1 - 1/2N) = (1 - \frac{1}{4}) = \frac{3}{4}$  of that on the mainland. Since heritability is proportional to heterozygosity, this bottleneck at foundation reduces evolutionary potential by about one-quarter through its impact on  $h^2$ .

The selection differential ( $S$ ) will be reduced in the Isle Royale population due to inbreeding depression. With an initial inbreeding coefficient of 25%, the reduction in lifetime production of offspring reaching sexual maturity is likely to be about 50% (Frankel & Soulé [1981](#)). We will assume that the mainland population has 30 pups per pair over five years and the island population only 15. If the population size is stable, then one pair of pups from each pair contributes to the next generation. This represents 6.7% of the mainland population breeding and

13.3% of the island population, giving selection differentials (S) of 1.9 and 1.6, respectively (using tables in Becker 1984). Thus, the island population would have a 16% lower selection differential than the mainland population.

If the mainland population of wolves is 5000 breeding individuals and the Isle Royale population 25 potentially breeding individuals per generation, then, with a conservative  $N_e/N$  ratio of 0.2, the effective sizes are 1000 and 5. Consequently, the loss of genetic diversity for the mainland population will be very small (1/2000 per generation), and after 20 generations, only 1% of the initial heterozygosity will be lost. However, with only 5 effective individuals breeding on the island, 10% of the existing genetic variation will be lost each generation. From Equation 15.5, the response to selection  $R$  in the Isle Royale population over the first five generations, will be

$$\begin{aligned} R_1 &= \frac{3}{4} h^2 \times 1.6 = 1.2 h^2 \\ R_2 &= \frac{3}{4} h^2 \times 1.6 \times \left(1 - \frac{1}{10}\right) = 1.08 h^2 \\ R_3 &= \frac{3}{4} h^2 \times 1.6 \times 0.9^2 = 0.97 h^2 \\ R_4 &= \frac{3}{4} h^2 \times 1.6 \times 0.9^3 = 0.87 h^2 \\ R_5 &= \frac{3}{4} h^2 \times 1.6 \times 0.9^4 = 0.79 h^2 \end{aligned}$$

The cumulative response over the five generations will be  $4.91 h^2$  in the Isle Royale gray wolf, while that for the large mainland population, calculated as above, will be  $9.5 h^2$ .

Thus, response in the Isle Royale population will be only  $4.91/9.5 = 52\%$  that of the mainland population.

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The arguments above and in Example 15.1 emphasize the importance of expanding the population sizes of endangered species to minimize inbreeding and loss of genetic variation and to improve their ability to evolve in response to environmental changes.

## Long-term retention of single-locus genetic diversity

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Effective population sizes of  $10^5$ – $10^6$  are required to retain single-locus diversity due to the balance between mutation and drift

Some loci, such as self-incompatibility loci in plants, sex loci in Hymenoptera and MHC loci in vertebrates, are of such importance to survival that we must aim to retain their genetic diversity. Population sizes required to retain single-locus diversity are much larger than those for quantitative characters, as mutation rates for functional variants at single loci are only  $10^{-5}$ – $10^{-6}$ .

Based on mutation–drift equilibrium, Lande & Barrowclough (1987) suggested that effective population sizes of  $10^5$ – $10^6$  were required to retain single-locus diversity. These sizes are unattainable goals for most species (especially vertebrates) of conservation concern, given current habitat availability and conservation resources. Population sizes required to maintain diversity at loci subject to balancing selection (e.g. self-incompatibility, the sex locus in haplo-diploids and MHC loci) will be less than this, but may also be unattainable goals.

## Time to regenerate genetic diversity

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If genetic diversity is lost, it is only regenerated very slowly by mutation, with recovery of original levels taking hundreds to thousands of

generations

Loss of genetic diversity would not be of great concern if it were regenerated rapidly by mutation. However, times to regenerate genetic diversity are very long, as mutation rates are low ([Table 15.1](#)). Single-locus diversity with a mutation rate of  $10^{-5}$ – $10^{-6}$  per generation takes 100 000 to 10 million generations to regenerate. Quantitative genetic variation has an estimated mutation rate of  $10^{-3} V_E$  per generation, and requires only 100–1000 generations to regenerate – still about 2600–26 000 years for elephants! Clearly, we cannot rely on mutation to regenerate genetic diversity in threatened species, at least in time spans of conservation concern. The implication is that every effort must be made to prevent loss of current genetic diversity in the first place.

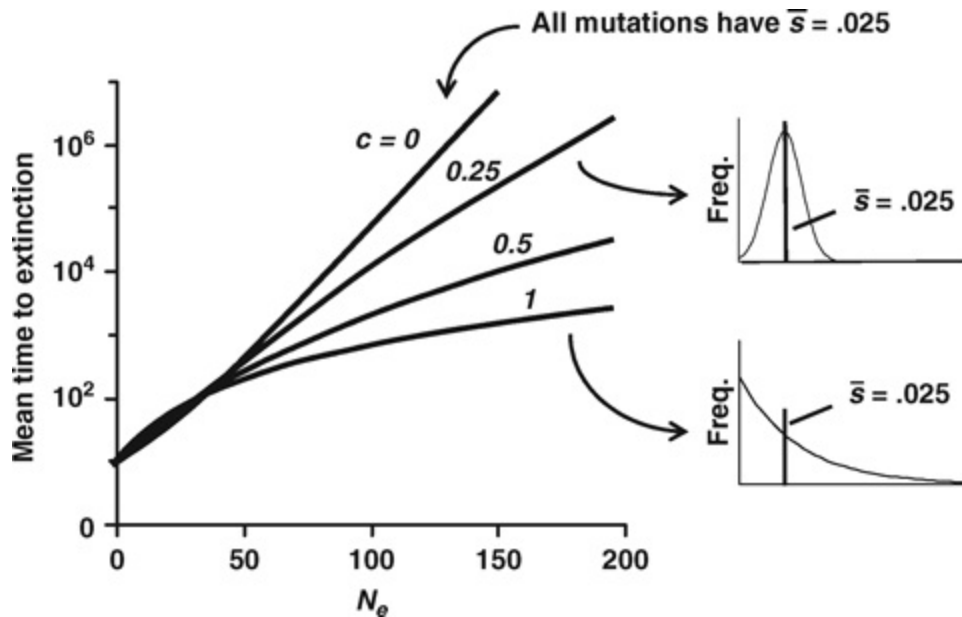
## Avoiding accumulation of new deleterious mutations

Some mildly deleterious mutations are fixed by chance in small populations and result in reduced reproductive fitness. However, mutational accumulation appears to be a much lesser threat than inbreeding and loss of genetic diversity

Deleterious mutations of small effect become effectively neutral in small populations and a proportion of them will be fixed by chance ([Chapter 9](#)). This may result in reduced reproductive fitness, leading to a decline towards extinction (**mutational meltdown**) (Lande [1995](#); Lynch *et al.* [1995a](#)).

Since mutation rates are low, they accumulate slowly. Thus, their threat is only evident in the long term. Nonetheless, there is evidence that mutational accumulation can cause declines in asexual populations (de la Peña *et al.* 2000; Paland & Lynch 2006). In contrast, recombination in outbreeding sexual populations allows natural selection to more effectively oppose fixation of deleterious alleles, as some progeny have higher reproductive fitness than that of their parents.

The significance of mutational accumulation in sexually reproducing populations is controversial. Lande (1995) suggested that effective population sizes below 1000 would suffer serious declines from the random fixation of new, mildly deleterious mutations (Fig. 15.2). However, Lynch *et al.* (1995a) argued that it would only cause problems in populations with sizes  $N_e < 100$ , while Charlesworth *et al.* (1993) did not consider this factor to be a serious threat unless  $N_e < 12$ . The effects of mutational accumulation on population persistence are mild when most mutant alleles have large deleterious effects, but serious when there are many mutant alleles with small deleterious effects (Garcia-Dorado 2003). Favourable and compensatory mutations reduce the impacts of deleterious mutations on fitness and extinction risk (Lande, 1998; Whitlock, 2000; Estes & Lynch 2003; Whitlock *et al.* 2003). There is no consensus about the rate and distributions of effects of new mutations (Keightley and Lynch, 2003).



**Fig. 15.2** Mean times to extinction (in generations) as a function of effective population size due to fixation of new mutations (after Lande 1995). The values of  $c$  reflect different assumptions about the distribution of effects of new mutations, as illustrated.

Experimental evidence indicates that mutational accumulation in naturally outbreeding species is of minor importance in the time frame of most conservation concern (100–200 years). No evidence of mutational accumulation was found in fruit fly populations maintained for 45–50 generations at effective sizes of 25, 50, 100, 250 and 500 (Gilligan *et al.* 1997), or in 12 asexual yeast populations maintained with  $N_e \sim 250$  for 2900 generations (Zeyl *et al.* 2001). Even in 12 yeast populations with elevated mutation rates (200 times normal) there was only one extinction over 2900 generations. Nematode populations of  $N = 1$ , with normal mutation rates, lost less than 1% of their fitness per generation (Vassilieva *et al.* 2000; Estes *et al.* 2004). In practice, inbreeding depression is likely to cause extinction before mutational accumulation is a serious issue. For example, while no effects of mutational accumulation were detected, inbreeding depression was evident in the populations of  $N_e = 25$  and 50 studied by Gilligan *et al.* (1997) (Woodworth *et al.* 2002).

## Genetic goals in the management of wild populations

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Few management programs for endangered species in the wild include genetic objectives

We are aware of only a few management plans for endangered species in the wild where genetic objectives are defined. In the golden lion tamarin, the objective is to retain 98% of genetic diversity for 100 years, corresponding to an effective size of about 400 (Example 15.2). Currently there are about 2100 individuals (1600 wild animals, 400 from reintroductions and 500 in captivity). Unless the  $N_e/N$  ratio exceeds 0.19 for all animals, or 0.25 for wild animals, which is unlikely, the genetic goal is not being achieved. Lack of available habitat for population expansion is the primary constraint.

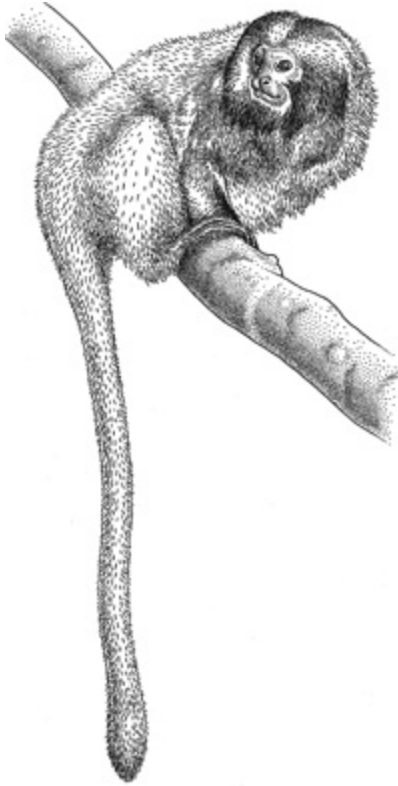
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### **Example 15.2 Effective population size required to retain 98% of genetic diversity for 100 years in the golden lion tamarin.**

To obtain the required population size we use [Equation 11.1](#):

$$\frac{H_t}{H_0} = \left(1 - \frac{1}{2N_e}\right)^t \sim e^{-t/2N_e}$$





Golden lion tamarin

Substituting  $H_t/H_0 = 0.98$  and  $t = 100/6 = 16.7$  (tamarins have a generation length of six years), we obtain

$$0.98 = e^{-16.7/2N_e} = e^{-8.333/N_e}$$

after taking natural logarithms (ln) of both sides, we obtain

$$\ln 0.98 = -\frac{8.33}{N_e}$$

and

$$N_e = \frac{-8.33}{\ln 0.98} = 412$$

Thus, the effective size required to retain 98% of genetic diversity for 100 years in wild golden lion tamarins is an effective size of over 400.

In practice, the  $N_e$  needed to reach this goal is estimated using computer simulations, taking into consideration all the relevant life and

population structure details (Holst *et al.* 2006).

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## Recovery targets for population sizes used to de-list species

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Population size targets for de-listing endangered species vary, but are generally smaller than genetic considerations would recommend

Table 15.3 lists the target sizes required for de-listing a range of endangered species. These are based on many considerations, but frequently ignore genetic concerns. While most target sizes are in the thousands, they are generally less than genetic arguments require, based on a  $N_e/N$  ratio of 0.1. The numbers ( $N$ ) for peregrine falcons and California condors are particularly alarming, at about 900 and 450. The peregrine falcon and bald eagle have now been de-listed, as they exceeded their target population sizes.

**Table 15.3** *Population size targets specified for de-listing a range of endangered species*

Species	Target size for de-listing	Reference
<b>Mammals</b>		
Asian rhinoceros	2500 ( $N_e > 500$ ) in 10+ populations	1
Black-footed ferrets	1500 adults in 10+ populations	2
Sea otter	2650	3
<b>Birds</b>		
Attwater's prairie chicken	5000 in three different areas	4
Bald eagle	3900 pairs	5
California condor <sup>a</sup>	2 x 150 wild + 150 captive	6
Peregrine falcon	456 breeding pairs	7
Red-cockaded woodpecker	5 populations of 500 = 2500	8
<b>Plants</b>		
Lakeside daisy	1000 plants	9

<sup>a</sup> Downlisting from endangered to threatened.

*References:* 1, Foose *et al.* (1995); 2, Clark (1994); 3, Ralls *et al.* (1996); 4, Bowdoin & Williams (1996); 5, Millar (1999); 6, Ralls *et al.* (2000); 7, Mesta (1999); 8, Kulhavy *et al.* (1995); 9, Dem Mauro (1994).

## Genetic goals in management of captive populations: a compromise

Captive populations of endangered species are usually managed to retain 90% of their genetic diversity for 100 years

As there are far fewer available resources than would be required to maintain all the species deserving captive breeding, the current compromise

is to manage such programs to conserve 90% of natural genetic diversity for 100 years. The 100-year time frame is based on the presumption that wild habitat will become available following the predicted human population decline in 100–200 years (Soulé *et al.* 1986). An approximate expression for the required size can be obtained using Equation 11.1, as follows:

$$\frac{H_t}{H_0} = \left(1 - \frac{1}{2N_e}\right)^t \sim e^{-t/2N_e}$$

Upon taking natural logarithms, substituting 0.9 for  $H_t/H_0$ ,  $100/L$  for  $t$  (where  $L$  is generation length in years), and rearranging, we obtain

$$N_e = \frac{475}{L} \quad (15.7)$$

Consequently, the required size is inversely proportional to the generation length for the species (Fig. 15.3). A range of examples is given in Table 15.4 where the effective size required to maintain 90% of the original heterozygosity is 475 for a species with one generation per year, 18 for Caribbean flamingos (generation length = 26 years) and 1759 for the white-footed mouse (generation length = 14 weeks). This is one of the few circumstances where long-lived species are at an advantage.

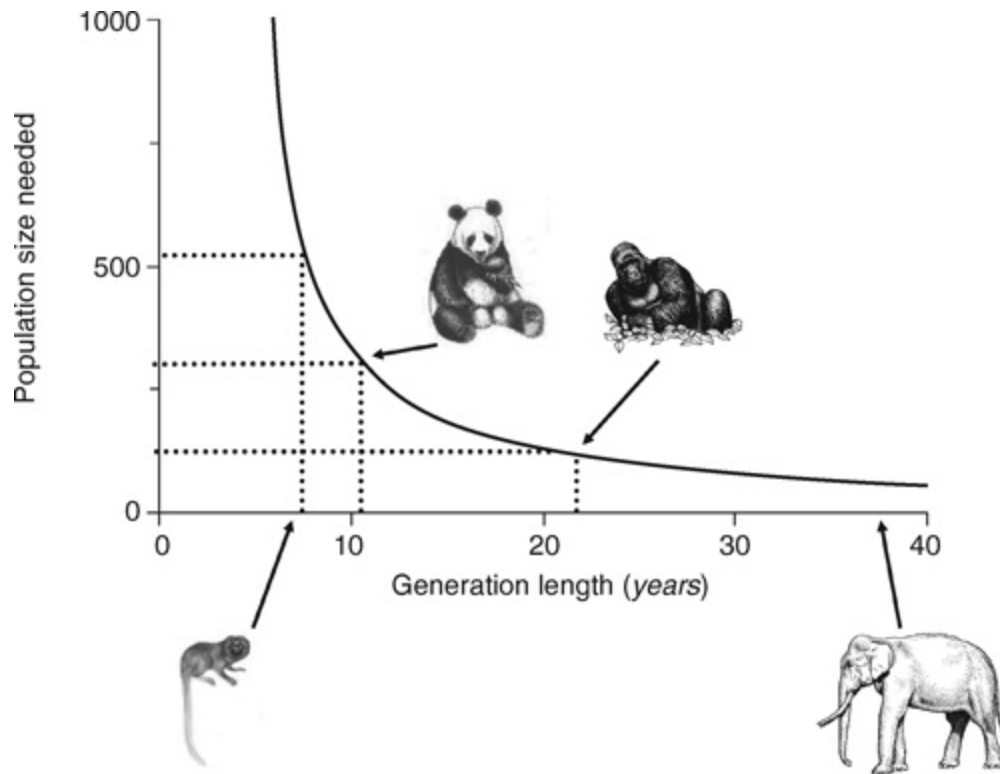
**Table 15.4** *Effective population sizes required to retain 90% of original heterozygosity for 100 years in species with different generation times*

Species	Generation time (yrs)	$N_e$
White-footed mouse	0.27 <sup>a</sup>	1759
Striped grass mouse	0.75	633
Houston toad	1	475
<i>Partula</i> snail	5 <sup>b</sup>	95
Brush-tailed bettong	6	79
Bullfrog	7	68
Siberian tiger	7	68
Nyala	8	58
Arabian oryx	10	48
African black-necked cobra	10	48
Mauritius pink pigeon	10	48
Indian rhinoceros	18	26
Caribbean flamingo	26	18
White-naped crane	26	18

<sup>a</sup> Millar & Zammuto (1983).

<sup>b</sup> P. Pearce-Kelly (pers. comm.)

Source: Modified from Conway (1986).



**Fig. 15.3** Effective population size required to retain 90% of genetic diversity for 100 years for species with different generation lengths, based on  $N_e = 475/L$ .

While maintenance of 90% of genetic diversity for 100 years is a reasonable practical compromise, it is unlikely that all the species requiring captive breeding can be accommodated. Species are being maintained with lesser goals (and smaller sizes) as a consequence of shortage of resources.

The cost of the 90% compromise is increased inbreeding and reduced reproductive fitness. From [Chapter 12](#):

$$F = 1 - \frac{H_t}{H_0}$$

The accepted 10% loss of heterozygosity corresponds to an increase of  $F$  of 10%, with consequent inbreeding depression. In captivity this will reduce juvenile survival on average, by ~15% and total fitness by ~25% ([Chapter 13](#)). The fitness costs are likely to be much greater if species are subsequently

reintroduced into harsher wild environments (Armbruster & Reed 2005). Thus, captive breeding programs are balancing the cost of permitting a moderate degree of inbreeding over 100 years against the benefits of maintaining extra endangered species within the limited resources available.

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Population sizes required to retain 90% of genetic diversity for 100 years are affected by small founder numbers, different rates of population growth and by  $N_e/N$  ratios less than unity

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The discussion above ignores the bottleneck associated with founding captive populations and assumes that population numbers can immediately be raised to the desired size. Captive populations typically have few founders and grow slowly to their final sizes. Together, these factors lead to greater than predicted losses of genetic diversity early in the captive breeding program. Consequently, effective population sizes required to retain 90% of genetic diversity for 100 years are typically greater than given above. [Box 15.2](#) illustrates the required size for the captive population of golden lion tamarins.

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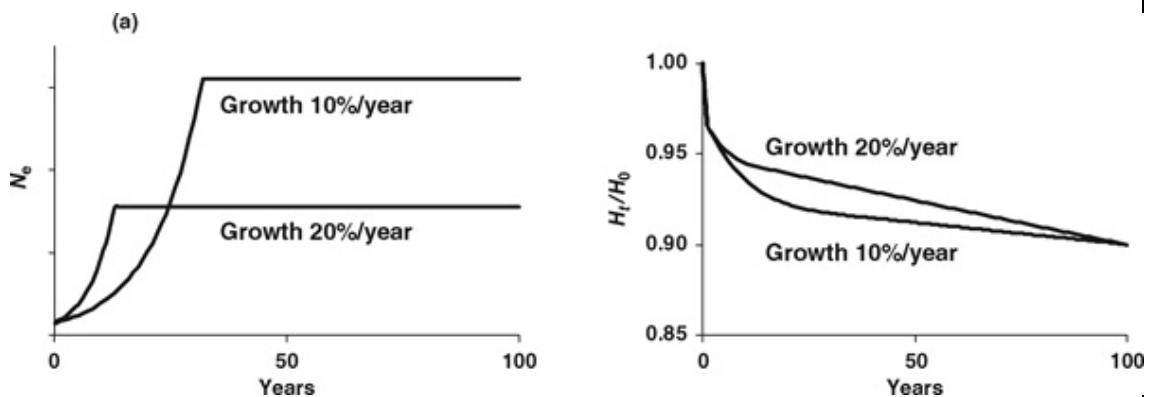
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**Box 15.2 Determining the effective population size required to maintain 90% of genetic diversity for 100 years in golden lion tamarins**

Calculating the  $N_e$  required to maintain 90% heterozygosity for 100 years (the target effective population size) requires knowledge of generation length, number of founders and potential population growth rate (to

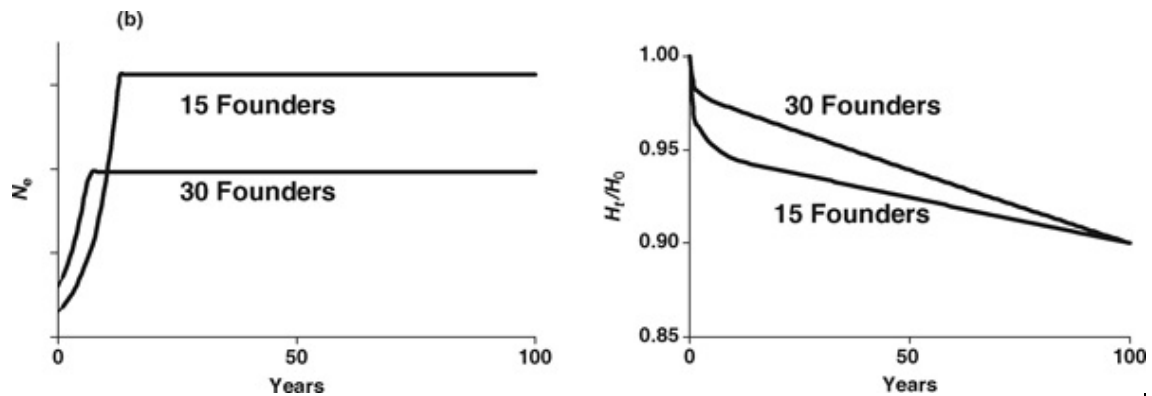
model the growth of the population from the founders to the required  $N_e$ ).

Golden lion tamarins have a generation length (average age of reproduction) of about six years. They started with about 15 effective founders and the population can grow as rapidly as 20% per year, although a more realistic value might be 10% per year. The effects of different population growth rates are illustrated in figure (a). The upper left panel shows population ( $N_e$ ) increase over time for the two different growth rates. The upper right panel shows the loss of heterozygosity over time. The slower-growing population needs more time to reach a larger final size ( $N_e = 312$ ) than the faster-growing population ( $N_e = 155$ ). This arises from the slower-growing population losing more heterozygosity early in its history than does the more rapidly growing population and therefore needs to reach a larger final size to compensate for the early loss.



The number of founders also affects the required  $N_e$ . Figure (b) illustrates this bottleneck effect on the target population size for two populations with the same growth rate (20%/year) but with 15 versus 30 founders. The former population requires a larger final size ( $N_e = 155$ ) than the latter ( $N_e = 98$ ), due to loss of heterozygosity during its more severe founding bottleneck.





Genetic management of captive populations by planned matings allows  $N_e/N$  ratios to be higher than those in the wild, typically about 0.3. From the example above, the population size required to maintain 90% heterozygosity for 100 years in golden lion tamarins might range from 326 for a well-founded and rapidly growing population, to 1040 for a slower-growing population founded with small numbers. The current captive population is being managed at about 500 individuals ([Box 20.1](#)).

Shortage of space to house the desired population size is most serious for small mammals with short generation lengths. Black-footed ferrets require over 19 000 individuals to maintain 90% of their heterozygosity for 100 years. Their generation length is about 2.5 years and the population started with only eight effective founders.

When requirements exceed available resources, the program must accept that either the population will become more inbred than 10%, or that the time frame for management is shorter than 100 years. For example, with black-footed ferrets, the captive management objectives were adjusted to 90% retention of heterozygosity for 50 years. The shorter time frame was justified because a reintroduction program was implemented within the first three generations (Ballou & Oakleaf [1989](#)). Detailed treatment of the genetic issues of captive breeding and reintroduction is given in [Chapters 19](#) and [20](#).

## The fallacy of small surviving populations

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The persistence of a few small populations following severe bottlenecks does not contradict the conclusions that inbreeding and loss of genetic diversity are normally deleterious and that population sizes in the thousands are usually required for long-term viability

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Several species have survived bottlenecks of a few individuals, while others have survived at small sizes for considerable periods of time (Craig 1994) (although many more have gone extinct). Examples include the northern elephant seal, Chatham Island black robin, Hawaiian crow, Mauritius kestrel, Mauritius pink pigeon, Seychelles robin and Socorro Island red-tailed hawk (Chapter 13). Consequently, some authors have questioned the importance of inbreeding and loss of genetic diversity in population viability (see Craig 1994; Elgar & Clode 2001).

It is fallacious to argue from a few surviving bottlenecked populations that bottlenecks are not a cause of extinctions (Soulé 1987). This is akin to arguing that ‘granddad smoked 30 a day and lived to 80, so smoking doesn’t contribute to cancer’ (Sunnucks 2000). While some bottlenecked populations have survived, we must not ignore those (the majority?) that have failed to do so (Laikre *et al.* 1997), or those that are trapped in the extinction vortex (Fig. 2.2).

The effects of inbreeding have a large stochastic element as they depend on the chance fixation of deleterious alleles (Chapter 13). About 5–20% of populations deliberately inbred to  $F > 90\%$  survive in benign laboratory conditions (Frankel & Soulé 1981). The small surviving populations of wildlife are not a random sample. They are analogous to the few highly

inbred lines that survive, or the few heavy smokers who live long lives. Where unbiased samples have been studied, they revealed that surviving small inbred populations had reduced fitness ([Chapter 13](#)). Most of these small inbred wild populations will be highly vulnerable, as the extinction proneness of island populations attests (Frankham [1998](#)).

Many remnant populations have benefited from intensive management. For example, northern elephant seals only recovered following protection from hunting, Mauritius kestrels following banning of DDT use, European bison herds following protection from hunting and provision of supplementary feeding during winter and Seychelles warblers following translocation to nearby islands with greater food supplies.

Some island populations of threatened species survive as they lack the predators or diseases that drove mainland populations to extinction. For example, black-footed rock wallabies are being decimated on mainland Australia by introduced red foxes, but foxes are absent from the islands where wallabies persist. However, the island populations show evidence of severe genetic problems (loss of genetic diversity and inbreeding depression: Frankham [1998](#); Eldridge *et al.* [1999](#); Groombridge *et al.* [2000](#)).

We should not let the occasional persistence of genetically impoverished populations mislead us. We wish to conserve the majority of threatened species, rather than permitting extinction of many species and conserving only a few exceptional cases.

The conclusions in this chapter set minimum population sizes only for genetic viability. In natural populations, demographic and environmental stochasticity and catastrophes combine with genetic factors to elevate extinction risks ([Chapter 22](#)).

## Summary

1. Since resources for maintaining threatened species in both the wild

and captivity are limited, attempts have been made to define minimum sizes for genetically viable populations.

2. The size of populations required to avoid inbreeding depression is greater than  $N_e = 50$ . All small populations of outbreeding species are likely to eventually suffer inbreeding depression. Mean times to extinction (in generations) due to inbreeding depression in captivity approximate the effective size of populations.
3. To retain evolutionary potential, effective population sizes of 500–5000 have been recommended, corresponding to actual population sizes of 5000–50 000 in the wild.
4. Essentially all threatened species have reduced long-term evolutionary potential, as this is related to  $N_e$ , genetic diversity and reproduction rate.
5. The size of population required to avoid accumulation of new deleterious mutations is unclear and controversial, but this factor appears to be much less important than inbreeding.
6. Current population sizes for most threatened species in the wild and in captivity are typically too small to avoid genetic deterioration within time frames of conservation concern.
7. Due to space and resource constraints, captive populations of endangered species are typically managed to retain 90% of the genetic diversity for 100 years.
8. The existence of a few cases where small populations have survived does not contradict the general rule that small populations are at a high risk of extinction.

### **Further reading**

Franklin (1980) Classic paper that estimated sizes required for population to be genetically viable for conservation.

Franklin & Frankham (1998) Response to Lande's (1995) estimate that  $N_e = 5000$  is required to retain evolutionary potential.

Lande (1995) Revised the  $N_e$  required to maintain evolutionary potential from 500 to 5000. Also considered the likely impact of mutational

accumulation.

Soulé *et al.* (1986) Proposed the captive breeding goal of retaining 90% of genetic diversity for 200 years (later modified to 100 years).

Soulé (1987) *Viable Populations for Conservation*. Book with papers considering different aspects of 'How large', both genetic and ecological.

Whitlock *et al.* (2003) Review of mutation accumulation models and data.

## Software

PMx: Software used to estimate  $N_e$  required to maintain 90% of genetic diversity for 100 years (Pollak *et al.* 2009). [www.vortex9.org/pmx.html](http://www.vortex9.org/pmx.html)

## Problems

- 15.1 Evolutionary potential. For some characters, estimates of  $V_m = 3 \times 10^{-3} V_E$  have been obtained. If the character has a heritability of 15%, what  $N_e$  is required to preserve short-term evolutionary potential?
- 15.2 Evolutionary potential. If 90% of mutations are deleterious, what  $N_i$  is required under the conditions in Problem 15.1?
- 15.3 Maintenance of genetic diversity. Derive the expression for the effective size required to maintain 95% of genetic diversity for 100 years.
- 15.4 Maintenance of genetic diversity. How large does a bank vole population, with three generations per year, need to be to retain 90% of its genetic diversity for 100 years?
- 15.5 Maintenance of genetic diversity. How large a population is required for the endangered palm cockatoo (generation length of 20 years) to retain 90% of its genetic diversity for 100 years?
- 15.6 Maintenance of genetic diversity. How many elephants (26 years per generation) and deer mice (three generations per year) are needed to maintain (a) 95% of the diversity for 100 years? (b) 90% of

original diversity for 50 years?

- 15.7** Evolutionary potential. The population size goal for the California condor recovery program is 150 birds in each of three populations (two wild and one captive). This is substantially less than the  $N_e$  needed to maintain evolutionary potential. Will the California condor go extinct because of this?

## **Section III From theory to practice**

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In Section III we apply the conclusions on evolutionary genetics of populations and the deleterious genetic consequences of population size reduction (from [Sections I and II](#)) to the genetic management of threatened populations and species.

### **Taxonomic uncertainties and management units**

A critical first step in conserving a species is to gain a clear understanding of its taxonomy. Is the population of interest a unique species? Does it actually consist of multiple cryptic species? Or is it simply another population of a common species? Without this knowledge endangered species may be denied protection, or resources wasted on populations of common species. The use of genetic techniques to assist in resolving taxonomic uncertainties is described in [Chapter 16](#). To do this we must first define what is meant by a species and consider briefly how speciation occurs. Populations within species may be so distinct that crosses suffer reduced reproductive fitness (outbreeding depression). The chapter concludes by considering means for defining management units within species.

### **Management of wild populations**

The genetic management of wild populations is considered in [Chapter 17](#). Typically wild population management is concerned with increasing population sizes and alleviating the effects of population fragmentation.

Species with inadequate gene flow among population fragments will suffer insidious processes of inbreeding depression, loss of genetic diversity and eventually population extinctions, unless gene flow is re-established. Sadly, there continues to be only limited activity in this area. Genetic management of fragmented populations represents the greatest unmet genetic challenge in conservation biology. The chapter also addresses the different management regimes required for species varying in breeding systems.

## **Conservation genetics of invasive species**

Invasive species represent one of the major threats to endemic species through predation, parasitism, infectious disease, hybridization and competition. There are important genetic issues involved in the likelihood of species becoming invasive, in the impacts of invasive species on the evolution of native species and in relation to control of invasive species. These issues are addressed in [Chapter 18](#), a new chapter in this edition.

## **Captive management and reintroduction**

A large and increasing number of species have their numbers reduced to the point where they are at risk of extinction unless there is benign human intervention. In many cases the only way to save these species is by ***ex situ* conservation**, i.e. by preserving species away from their natural habitats. About 4000–6000 species of terrestrial vertebrates alone require captive breeding to save them from extinction. *Ex situ* conservation takes many forms, including captive breeding in zoos, wildlife parks and aquaria, conservation of plants in botanical gardens and arboreta, seed storage, and cryopreservation of animals, plants, cell cultures and gametes. However, space, numbers, costs, continuity and adaptation to captivity limit the value of *ex situ* conservation. It is most appropriate for large vertebrates and for plants. [Chapter 19](#) covers the genetic management of captive populations. Maintaining viable populations with the potential for reintroduction to the wild is an objective of many such programs. [Chapter 20](#) considers the genetic management of captive populations to minimize genetic changes that



adversely affect reintroduction success, as well as the genetic issues involved in the reintroduction process itself.

## **Use of molecular genetics in forensics and to understand species biology**

Molecular genetic methods are used in forensics to detect illegal hunting and trade in endangered species. These methods are also helping us understand the basic biology of species, knowledge necessary for their conservation ([Chapter 21](#)). For example, genetic markers can be used for resolving paternity, sexing birds and marine mammals, determining mating systems, population structure, dispersal rates, population size and diet, and detecting disease and hybridization with other species.

## **The broader context**

Wild populations in natural habitats suffer human impacts (habitat loss and degradation, over-exploitation, pollution and introduced species), and stochastic threats (demographic, environmental and genetic stochasticity, and catastrophes) that contribute to extinctions. [Chapter 22](#) discusses the combined impacts of all deterministic and stochastic threats, and so provides a connection between conservation genetics and broader issues in conservation biology. Stochastic computer projections (population viability analysis – PVA) is a major tool used for predicting the risk of extinction due to the combination of all threatening processes, as well as a research tool and a means for comparing management options for species recovery.

## Chapter 16 Resolving taxonomic uncertainties and defining management units

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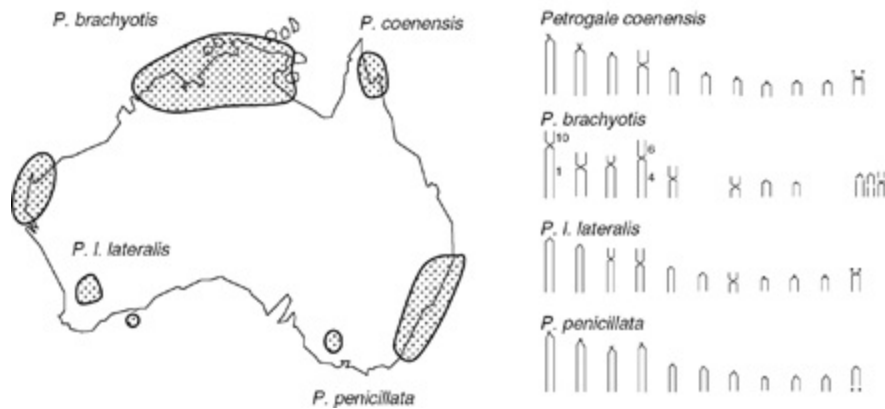
Taxonomic status must be accurately established to ensure that neither are endangered species denied protection, nor is effort wasted on abundant species. Genetic information assists in resolving taxonomic uncertainties and defining management units within species

### Terms

Allopatric, allotetraploid, amphidiploid, autotetraploid, biological species concept, conspecific, DNA barcoding, evolutionarily significant units (ESU), genetic distance, lineage sorting, management unit, maximum likelihood, molecular operational taxonomic units (MOTU), monophyletic, outbreeding depression, phylogenetic trees, polyphyletic, species, sibling species, speciation, sub-species, sympatric, taxa

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Rock wallabies in Australia, along with the chromosomes from a sample of species (after Eldridge & Close 1992). Many are endangered. Genetic methods have helped resolve taxonomic uncertainties in this group

## Importance of accurate taxonomy in conservation biology

Many inappropriate conservation decisions can be made if the status of a taxon is incorrect

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The taxonomic status of many taxa is unresolved. This is particularly true for lower plants and invertebrate animals, but also applies to large and obvious animals including deer, elephants, wallabies and wolves.

In conservation, many erroneous decisions may be made if the taxonomic status of populations is incorrectly assigned, including:

- unrecognized, endangered species may be allowed to become extinct
- endangered species may be denied legal protection while populations of common species, or hybrids between species, may be granted protection
- incorrectly diagnosed species may be hybridized with other species, resulting in reduced reproductive fitness
- populations that could be used to improve the fitness of inbred populations may be overlooked.

This chapter explores the rationale and methodologies for defining taxonomic status. It also considers practical definitions of management units within species, as the crossing of genetically differentiated populations can result in reduced population fitness (**outbreeding depression**).

Taxonomic uncertainties result from three causes:

- inadequate data,
- use of different species definitions, and
- populations presently part way through the evolutionary divergence of the speciation process.

Many species' descriptions are based on limited information on the geographic distribution of a small number of (usually morphological) traits of unknown genetic basis (Avice [1996](#)). Velvet worms (Phylum Onychophora) in Australia provide an extreme example. Only seven named species were recognized in a 1985 review, based on morphology. However, over 100 clearly diverged species have subsequently been identified using allozymes (Briscoe & Tait [1995](#)).



Velvet worm

Incorrect 'lumping' of several distinct species into one species has denied protection to endangered species. This has occurred for endangered Kemp's Ridley sea turtle, and *Helianthus exilis*, a sunflower from California (Rieseberg [1991](#); Bowen & Avice [1996](#)). The taxonomy of the threatened tuataras in New Zealand is uncertain and may include two or three distinct populations or taxa, one of which was at serious risk of extinction ([Box 16.1](#)).

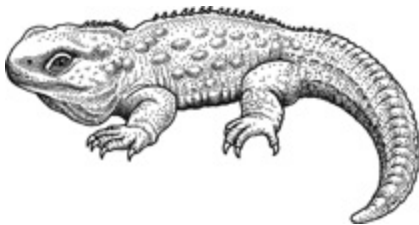
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**Box 16.1 Taxonomic uncertainty in tuataras and North American pumas and their conservation implications**

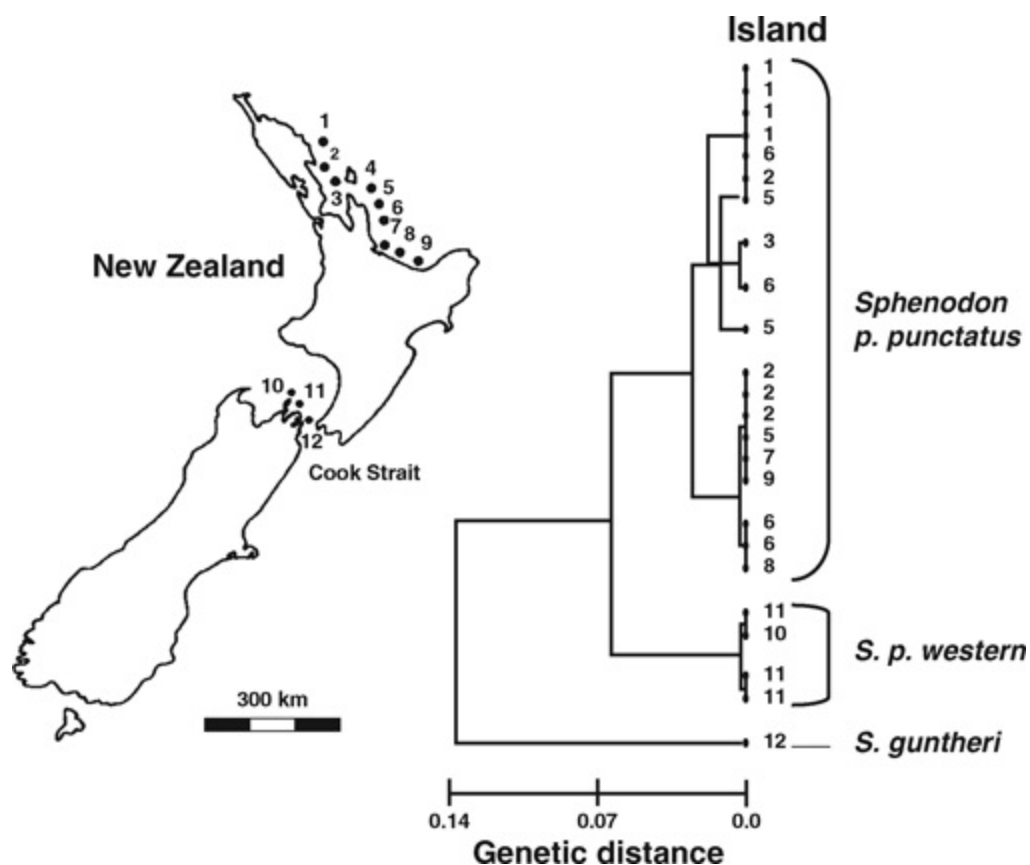
(Culver et al. 2000; Hay et al. 2003)

## Tuatara

The threatened tuataras in New Zealand are the only survivors of the ancient reptilian Order Rhynchocephalia, and were thought to be a single species. Studies of different island populations, using multiple allozyme loci, mtDNA, immunological comparisons and morphology, revealed two or three distinct groups. These are now being managed as separate conservation units, but the taxonomy remains uncertain.



Tuatara





Florida panther

### **North American pumas**

Mammalogists have recognized approximately eight morphological sub-species of North American pumas (also referred to as cougars, panthers and mountain lions), including the critically endangered Florida panther. However, microsatellite and mtDNA analyses found no significant differentiation among these populations, but did separate them from a number of South American sub-species.

Thus, the number of separate sub-species requiring conservation is reduced. Further, the controversial decision to augment Florida panthers with individuals from the Texas sub-species, to alleviate inbreeding depression, can now be recognized as a translocation.

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Conversely, ‘splitting’ of one species into two or more recognized taxa may lead to erroneous conservation decisions. Morphs within species have been incorrectly classified as distinct species in flightless New Zealand chafer beetles, the snow goose and in American and black oystercatchers (Emerson & Wallis [1994](#); Hebert *et al.* [2004](#)). Further, the eight recognized sub-species

of North American pumas, including the critically endangered Florida panther, are not genetically distinct ([Box 16.1](#)).

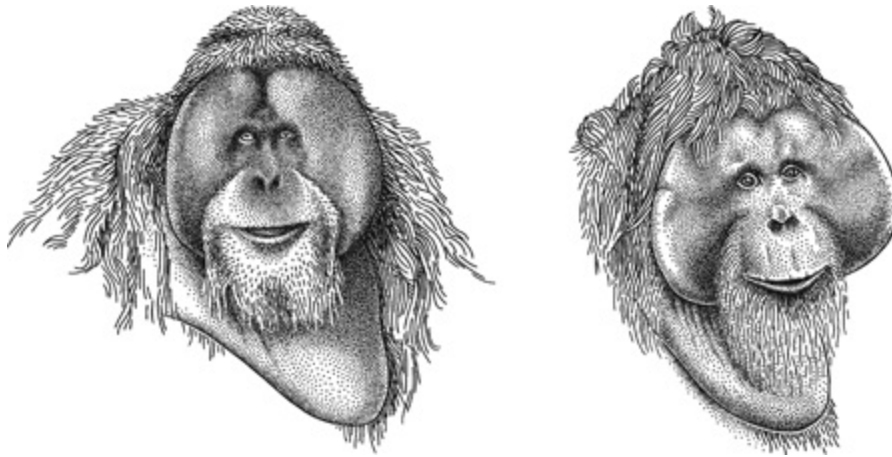
Hybridization between populations whose taxonomy is incorrect has created problems in some conservation efforts. For example, the last dusky seaside sparrow was unsuccessfully hybridized with an inappropriate seaside sparrow sub-species and became extinct, when subsequent genetic analyses revealed that a more closely related sub-species had been available ([Awise 2004](#)). Infertility in captive populations of dik-diks and owl monkeys was due to mixing of different chromosome races (probably undescribed species) ([Templeton 1986](#); [Ryder \*et al.\* 1989](#)). In addition, hybrids among common species have sometimes been misidentified as rare species deserving conservation ([Stuart & Parham 2007](#)).

Failure to recognize the degree of genetic differentiation between the Bornean and Sumatran sub-species of orangutans (possibly separate species) previously led to their hybridization in many zoos ([Box 16.2](#)). Conversely, two captive populations of Mexican wolf were kept separate from the one known small and inbred, ‘pure’ population until it was established that they had not hybridized with dogs, coyotes or gray wolves ([Hedrick & Frederickson 2008](#); [Chapter 21](#)).

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**Box 16.2 Genetic differentiation between Bornean and Sumatran orangutans: are they separate species? ([Xu & Arnason 1996](#); [Zhi \*et al.\* 1996](#); [Kanthaswamy \*et al.\* 2006](#); [IUCN 2007](#))**





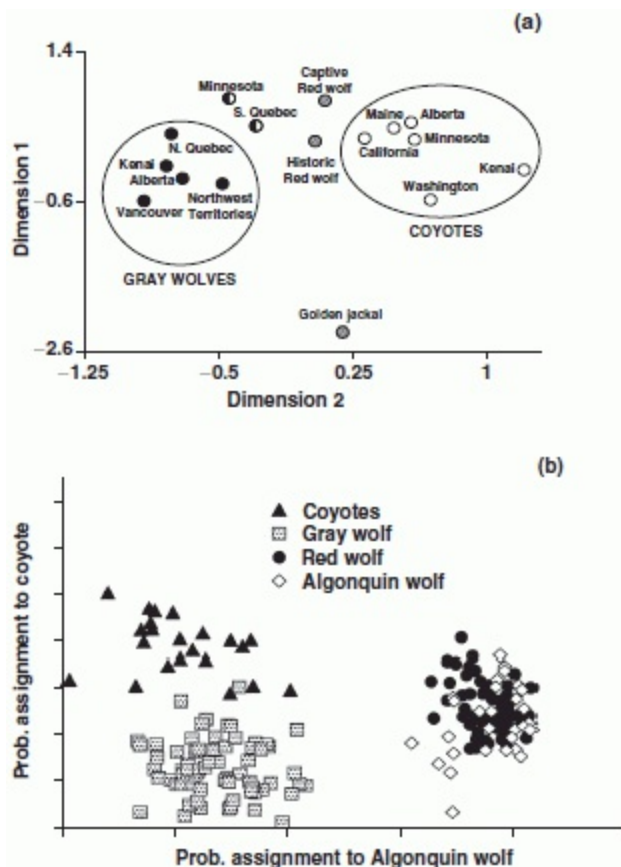
Bornean and Sumatran orangutans are (left and right images, respectively) restricted to their respective islands in Southeast Asia. As they are reported to differ in morphology, behaviour, chromosomes (a pericentric inversion difference, a reversed chromosomal segment), mtDNA sequences, protein coding nuclear loci and DNA fingerprints, and to show significant differences in microsatellite allele frequencies at eight of nine loci, they have been designated as sub-species or separate species. As they were thought to differ genetically by at least as much as do chimpanzees and bonobos, full species status for the two forms has been designated by some authors.

However, hybrids are found in many zoos and are viable and fertile in the  $F_1$  and  $F_2$  generations (Muir *et al.* 1998). Consequently, they are not distinct species according to the biological species concept. Further, a larger sampling has revealed that mtDNA sequences are not non-overlapping (not reciprocally **monophyletic**) as previously claimed and invalidate separate species status. They are not even strictly distinct evolutionarily significant units (see below), but do justify separate management. This illustrates the confusion created by different species definitions and indicates that designations may be changed by larger samplings of populations.

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The endangered red wolf in the southern USA has variously been considered to be a separate species, or a hybrid population between gray

wolves and coyotes. It is subject to a captive breeding and reintroduction program requiring substantial financial resources. Using microsatellites and mtDNA, Wayne and colleagues concluded that the red wolf was indeed a hybrid population (Fig. 16.1a). The red wolf contained no alleles that were not also present in gray wolves or coyotes. Subsequently, Wilson *et al.* (2000) have suggested that a small-bodied (Algonquin) wolf in eastern Canada is distinct from gray wolves and coyotes, but very similar to the red wolf (Fig. 16.1b).



**Fig. 16.1** Is the red wolf a distinct species, or a hybrid between gray wolves and coyotes? (Wayne 1996; Wilson *et al.* 2000). (a) Two-dimensional scaling of microsatellite data for red wolves, gray wolves and coyotes. The gray wolf circle encompasses non-hybridizing populations, while the Minnesota and southern Quebec populations have introgression from coyotes. The red wolves are intermediate between ‘pure’ gray wolves and coyotes, as expected if they are a hybrid population (from Wayne 1996). (b) Microsatellite data (below) and mtDNA sequence data both indicated clustering of Algonquin and red wolves as a distinct group from coyotes and gray wolves.

In practice, the taxonomy of particular groups of populations can usually be resolved with sufficient morphological, reproductive and genetic data (chromosomes, microsatellites, allozymes, DNA fingerprints and mtDNA). However, to appreciate this use of genetic markers, we must first review what is meant by a biological species, what we seek to conserve and how populations differentiate and speciate.



Gray wolf

## What is a species?

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There is no universally accepted definition of species

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Confusingly, there are at least 22 species definitions (Claridge *et al.* [1997](#)). These range from definitions based on morphology, ecology and genetics to definitions based on biological characteristics, evolutionary histories and phylogeny. Some definitions can, and have, classified the sexes of one species as separate species. The most appropriate definitions for conservation purposes are based on evolutionary units and gene flow.

Most named species have been delineated, on the basis of morphological characteristics, as groups of individuals that are distinct from all other groups. However, morphological definitions of species may have limited connection to genetics or evolution. Some groups of individuals initially appear morphologically indistinguishable, but are composed of two or more distinct species (**cryptic** or **sibling** species). For example, Chinese and Indian muntjac deer are morphologically similar, yet the former has 46 chromosomes, while the latter has 6 in males and 7 in females (Ryder & Fleischer [1996](#)).

---

Definitions of species generally recognize that individuals within an outbreeding species can exchange genetic material with each other, but not with individuals from different species

---

The **biological species concept** has been the most influential definition of species in population and evolutionary genetics and in conservation biology. This defines a species as a group of actually, or potentially, interbreeding individuals and natural populations that cannot interbreed with individuals from all other such groups (Simpson [1961](#); Mayr [1963](#)). This definition recognizes that individuals within a species can exchange alleles, while those from different species normally do not.

This definition provides a practical means for delineating species genetically. Populations of the same species will, if crossed, produce fertile offspring in the first and subsequent generations. Conversely, populations of different species will either fail to interbreed, or produce offspring with reduced survival or fertility. For example, lions and tigers can be hybridized, but their progeny are sterile.

The US Endangered Species Act is based on the biological species concept. However, it has encountered difficulties by excluding hybrids from conservation and by not dealing adequately with asexual forms. The biological species concept does not deal adequately with asexual and habitually self-fertilizing forms, becomes blurred for species that hybridize and has little relevance to classification of fossil specimens. Given these limitations, it is not surprising that the concept is controversial.

Lack of a universally recognized definition of species creates enormous difficulties in conservation biology, as many controversies are a result of using different definitions. For example, the phylogenetic species concept yielded 48% more species than the biological species concept for the same group of organisms (Marris [2007](#)). Concerns have been expressed about 'taxonomic inflation', as ever expanding numbers of species make it increasingly difficult to provide funding for conservation (Isaac *et al.* [2004](#); Meiri & Mace [2007](#)). Much of this increase is due to changing definitions of species, where sub-species are raised to full species rank.

In our view, a species definition for conservation must be based on evolutionary arguments, i.e. individuals within a species have linked evolutionary fates as they are capable of gene exchange, while different species have independent evolutionary trajectories. There is a degree of consensus on this view (Hey *et al.* [2003](#)). No matter what definition is used, there will always be difficult cases where taxa are part way along the path to speciation.

Currently, taxonomic classification is based upon an undefined mixture of morphological, behavioural, reproductive, molecular and chromosomal characteristics. Further, the majority of the species on the planet have not yet been identified, with around 1.75 million named and described species out of an estimated total of between 5 and 100 million species (Blaxter [2003](#); Kim & Byrne [2006](#)). There is a severe shortage of taxonomists and it typically takes substantial time to describe new species formally.

To address these problems, Tautz *et al.* (2002, 2003) proposed that DNA sequences be used as the universal reference standard for species identification. The reference specimen would be a DNA extract, with links to DNA sequence data, preserved individuals and other data. DNA taxonomy would speed up the process of classifying material, allow easy identification of juvenile forms and alleviate problems when linking information about species subject to name changes. The degree of DNA difference to delineate species still remains to be defined, but this would be done empirically. The proposal remains controversial, but has much merit. The following sections on **DNA barcoding** outline one proposal of this type. It is clear that approaches utilizing modern tools are required to address the crises in taxonomy, rather than continuing to rely upon the system devised by Linnaeus in the 1750s (Wheeler *et al.* 2004).

Widely accessible computer databases are being developed to overcome problems of accessing type specimens held in many institutions throughout the world and species descriptions spread throughout a plethora of journals. These include Zoobank (Polaszek *et al.* 2005; [www.iczn.org](http://www.iczn.org)), the Catalogue of Life (<http://www.catalogueoflife.org>) and the Encyclopedia of Life ([www.eol.org](http://www.eol.org)).

## DNA barcoding

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Proposals have been made to assign individuals to animal species based upon the sequence for a region of mtDNA

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To address the abovementioned taxonomic problems, Hebert *et al.* (2003a) proposed that the DNA sequences for a 648 base pair region of the mitochondrial cytochrome C oxidase 1 locus (CO1) be used (i) to assign

unknown individuals to species and (ii) to enhance discovery of new species (Blaxter [2003](#); Moritz & Cicero [2004](#)). This is referred to as a DNA barcode. mtDNA sequences in isolated populations differentiate more rapidly than nuclear loci, as their effective population size  $\sim 1/4$  that for nuclear genes, and their mutation rate is typically higher than for nuclear DNA. Further, primer sequences are readily available to amplify mtDNA sequences from most species.

While DNA barcoding is controversial, especially among taxonomists, it has gained considerable traction. It is estimated that the diversity of all animal life on Earth could be characterized by barcoding within 20 years at a cost of \$US1 billion (Hebert *et al.* [2003a](#)). It would take centuries to complete an encyclopedia of all life on Earth using current taxonomic methods (Wilson [2003](#)).

Promising results were obtained in pilot studies where 98% of 13 320 animal species pairs across 11 phyla showed greater than 2% sequence divergence (Hebert *et al.* [2003b](#)). Further, >95% of species in test assemblages of primates, birds, fish, gastropods, spiders, Lepidoptera and red algae were resolved (Lorenz *et al.* [2005](#); Hajibabaei *et al.* [2007](#)). Notably, mating trials in marine bryozoans have yielded close correspondence between CO1 barcode lineages and reproductive isolation in the context of minimal morphological change (Gómez *et al.* [2007](#)). A large number of new species have been identified using barcoding, especially in lesser studied taxa and in organisms from under-explored regions or habitats (Barber & Boyce [2006](#); Herre [2006](#); Gómez *et al.* [2007](#)).

However, DNA barcoding is controversial (Dasmahapatra & Mallet [2006](#)). Concerns include polymorphisms in common ancestral populations, male-biased gene flow, selection on mtDNA, gene flow following hybridization, and transfer of mtDNA loci to the nucleus (Moritz & Cicero [2004](#)) (Box [16.3](#)). Barcoding has not worked well in amphibians (Marshall [2005](#)) and it was less than 70% successful in correctly identifying species of Diptera (Meier *et al.* [2006](#); Whitworth *et al.* [2007](#)).

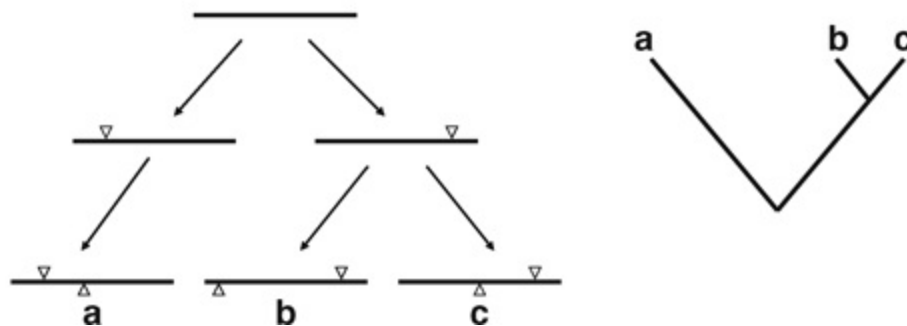
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## Box 16.3 Use of DNA sequence data to build phylogenetic trees

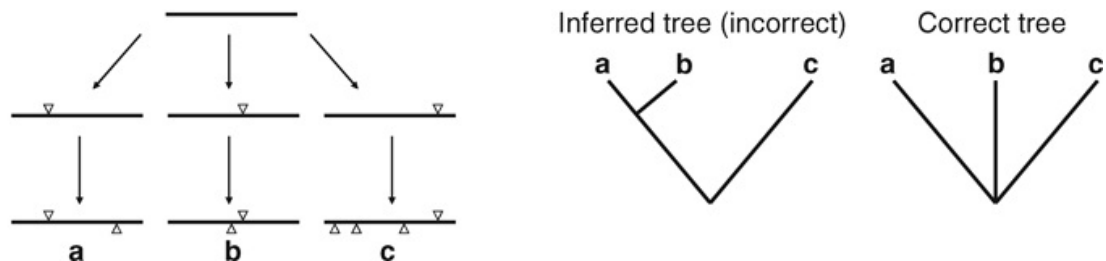
The following hypothetical examples indicate the conditions where DNA sequence data are likely to result in reliable phylogenetic trees, and those where they are not. In each example, an initial sequence evolves as we move down the page, so that mutations ( $\Delta$ , E) accumulate over time.

The correct phylogeny is only inferred in A with a monomorphic initial population and a constant rate of evolution in each lineage (branch). Conversely, incorrect phylogenies are likely when there is either unequal rates of evolution (B), or a polymorphic initial population (C).

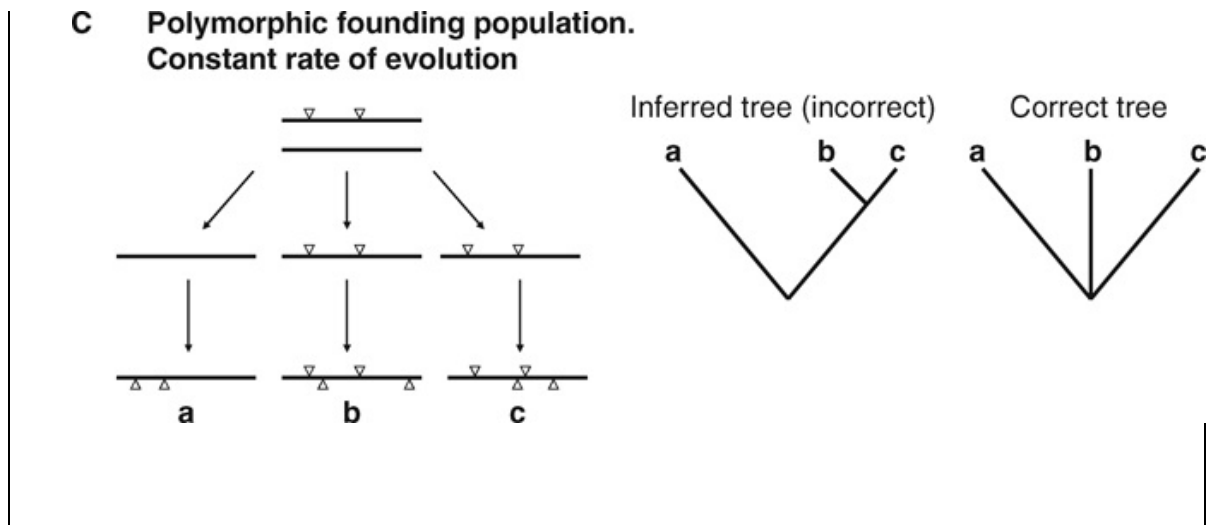
### A Monomorphic founding population. Constant rate of evolution



### B Monomorphic founding population. Unequal rate of evolution (higher in lineage c)







Unless they can be linked to previously identified species, the units identified by barcoding are referred to as **molecular operational taxonomic units** (MOTU) (Blaxter *et al.* 2005). A defined level of sequence difference is used as a cutoff for MOTU, and this is usually based upon calibration with known ‘good species’ in the taxon under study.

The CO1 locus is not suitable for barcoding in higher plants and some corals, as they have a much slower rate of mtDNA evolution than found in most animals. In plants, DNA sequences of a combination of nuclear and chloroplast regions have been proposed (Pennisi 2007).

## Sub-species

Sub-species are partially differentiated populations within a species

Threatened **sub-species** are frequently accorded legislative protection, and are the focus of substantial conservation effort. Sub-species are groupings of populations, within a species, that share a unique geographic range or habitat and are distinguishable from other subdivisions of the species by several genetically based traits (Avice & Ball [1990](#); O'Brien & Mayr [1991](#)). They are populations part way through the evolutionary process of divergence towards full speciation. Members of different sub-species rarely exhibit marked reproductive isolation. Crosses between the Bornean and Sumatran sub-species of orangutans produce fertile offspring with no apparent reduction in survival rates ([Box 16.2](#)).

The sub-species concept is more subjective than that of species and the reality of many sub-species have been questioned. In birds, only 36% of sub-species have been found to be phylogenetically distinct by possessing different mtDNA sequences (Phillimore & Owens [2006](#)).

## How do species arise?

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Speciation involves genetic divergence of populations until they are reproductively isolated

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Species arise in two major ways. The first is diversification, where a prior species gives rise to two or more descendent species. This occurs when populations genetically differentiate, and become reproductively isolated. Such speciation frequently involves at least partial geographic isolation. The second is gradual change within a lineage over time so that a previous species is named as a different species at a later time. In this chapter, we are concerned with the former – diversification of species. The process of

separation is not necessarily a continuous one and may involve divergence, interspersed with later gene exchanges before permanent separation, as appears to have occurred in the separation of chimpanzees and humans (Patterson *et al.* [2006](#)).

Speciation may also occur when hybridization between existing diploid species allows adaptation to a new environment, as has been documented in both plants and insects (Rieseberg *et al.* [2003](#); Schwarz *et al.* [2005](#); Mavárez *et al.* [2006](#)). Hybrid speciation without a change in chromosome number appears to be uncommon.

## Isolating factors

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Populations may become isolated by geographic features (allopatry), or a change (e.g. host shifts) within the same environment (sympatry)

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Physical isolating factors often result from geographic changes (mountain uprisings, desertification, river diversion, sea-level changes and continental drift) or from spread of organisms to novel territories. If the isolated populations become so genetically different that they do not interbreed upon secondary contact then speciation is termed **allopatric**. This is the most common form of speciation in animals (Coyne & Orr [2004](#); Gavrillets [2004](#)).

Speciation may also occur within the range of the ancestral species (**sympatric** speciation), for example when a parasitic species shifts from one host to another. Hawthorn flies that court and mate on the developing fruits of its host plants began to utilize apples in 1864 and cherries in 1960 (Howard & Berlocher [1998](#)). The somewhat different flowering times of these trees provided a temporal isolating mechanism and selective force

driving differentiation among populations on the different hosts. This may be a common form of speciation in parasites (Bush [1975](#)). Convincing cases of sympatric speciation are few, but are accumulating (Howard & Berlocher [1998](#); Hopkin [2006](#)).

Other mechanisms for speciation have been suggested. For example, White ([1978](#)) considered that chromosomal change was fundamental to speciation. Reduced fitness in hybrids produced at zones of sympatry of chromosomal races provides a driving force for the evolution of reproductive isolation. However, some related species have identical chromosome configurations and most species lack chromosomal races, so this is not a universal speciation mechanism and is generally considered to be uncommon.

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Reproductive isolation typically arises from adaptation to different environments

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Adaptation to distinct environments has a major role in leading to reproductive isolation (Coyne & Orr [2004](#); McKinnon *et al.* [2004](#); Nosil & Crespi [2006](#); Rieseberg & Willis [2007](#)). For example, three-spined stickleback fish in three isolated lake populations in western Canada independently evolved benthic (bottom dwelling) and limnetic (open water) forms with different sizes and diets, following glacial retreat 10 000 years ago (Rundle *et al.* [2000](#)). Benthics and limnetics from the same or different lakes showed reproductive isolation, but benthics from different lakes were not reproductively isolated, nor were limnetics from different lakes. Further, ecological divergence is positively associated with degree of reproductive isolation in eight data sets from plant, invertebrate and vertebrate taxa (Funk *et al.* [2006](#)). Conversely, evidence does not support a major role of genetic drift in speciation (Rice & Hostert [1993](#); Coyne & Orr [2004](#); Gavrillets [2004](#)).

Sexual selection may also be involved in speciation (Coyne & Orr [2004](#)).



Three-spined stickleback

## ‘Instant’ speciation

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Many plant species have been formed ‘instantly’ due to polyploidy

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Many plant species have arisen ‘instantly’ via **polyploidy** when the chromosome number increases, e.g.  $4n$  (tetraploid) (Ramsey & Schemske [2002](#)). These forms are substantially reproductively isolated from their progenitors. For example, the California redwood tree is a hexaploid with 66 chromosomes while its closest related living relative has  $2n = 22$  (Lewis [1980](#)).

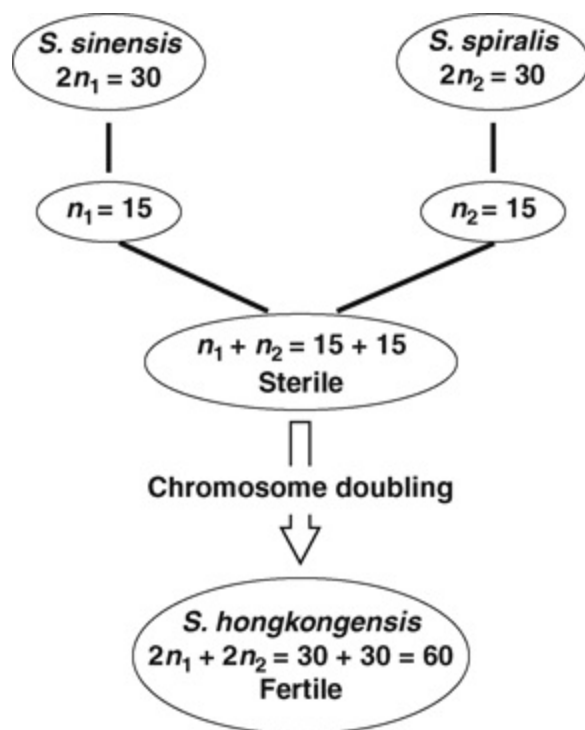


California redwood tree

Two forms of polyploidy occur: **autopolyploidy** and **allopolyploidy**.

Autopolyploids form by increasing the number of sets of chromosomes from within a species, presumably by production of diploid gametes. For example, there are both diploid ( $2n = 22$ ) and autotetraploid ( $4n = 44$ ) forms of the endangered grassland daisy in eastern Australia (Young & Murray 2000).

Allopolyploid species form by combining the complete chromosomal constitutions from two pre-existing species, as described for the rare allotetraploid Hong Kong lady's tresses orchid in Fig. 16.2. This form of polyploidy appears to be more common than autopolyploidy (Soltis *et al.* 2003).



**Fig. 16.2** ‘Instant’ speciation in the rare Hong Kong lady's tresses orchid through allopolyploidy (Sun 1996). The rare orchid *Spiranthes hongkongensis* arose from the hybridization of two diploid ancestors each with 30 chromosomes (*S. sinensis* and *S. spiralis*). The initial cross between the diploid progenitor species yielded a sterile hybrid. Following spontaneous doubling of chromosome numbers, or by production of diploid gametes, a fertile allopolyploid with 60 chromosomes resulted. *Spiranthes hongkongensis* formed only once, as almost all individuals have the same multilocus allozyme genotype. Crosses with its diploid progenitors yield

infertile triploids.



Hong Kong lady's tresses orchid

Genome doubling is now known to be widespread, perhaps characterizing most groups of organisms (Soltis *et al.* [2003](#)). Vertebrates have experienced at least one doubling. For example, polyploidy has been important in amphibians, and salmonids are ancient polyploids. Most (if not all), plants have undergone one or more episodes of polyploidization with roughly 2–4% of all speciation events in angiosperms and ~7% in ferns involving polyploidy.

## **Speciation is generally slow**

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Speciation takes thousands to millions of years, apart from that due to polyploidy

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Speciation generally occurs gradually over thousands to millions of years (Coyne & Orr [2004](#)). For example, some plant populations, such as sycamores and plantains in America and Asia that have been geographically isolated for at least 20 million years, still form fertile hybrids. The average

divergence times between mammals, frogs and birds still capable of producing viable hybrids are 2–3 million, 21 million and 22 million years (Coyne & Orr [2004](#)). On average, fruit flies (the best-studied group) take ~ 1.1 million years of separation to speciate in allopatry and 100 000 years in sympatry (Coyne & Orr [2004](#)). Some cases of speciation have been more rapid, but still involve many thousands of years. Hawaiian fruit flies have speciated in as little as 500 000 years. Cichlid fish in Lake Nabugabo, Africa have speciated within 4000 years and those in Lake Victoria within 100 000 years (Verheyen *et al.* [2003](#)). Sympatric speciation in the hawthorn fly (above) is one of the most rapid examples.

As evolution is an ongoing process, some populations will be observed part way through the speciation process. For example, rock wallaby populations in Australia (chapter frontispiece), many of which are endangered, show varying degrees of differentiation in morphology, chromosomes, allozymes and mtDNA, many exhibit only partial reproductive isolation and there are several hybrid zones (Eldridge & Close [1992](#), [1993](#)).

## **Delineating sympatric species**

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Genetic markers can be used to provide a definitive diagnosis of the taxonomic status of sympatric populations

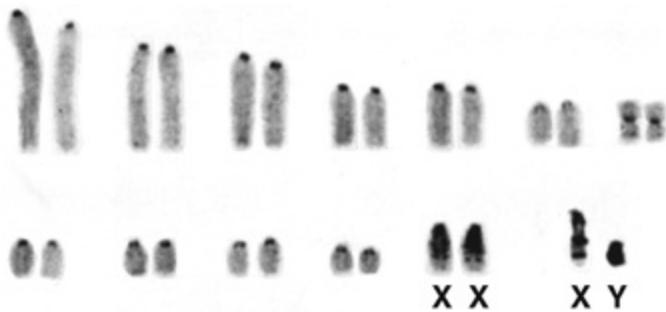
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Sympatric populations share the same or overlapping geographic distributions. According to the biological species concept, sympatric populations of the same species should exchange alleles, while distinct species sharing the same geographic region should not. Consequently, if any genetic marker shows lack of gene exchange, two sympatric populations



belonging to different species have been identified. In practice, several loci are required for such diagnoses. For example, two sympatric forms of potoroos (small marsupials) in southeastern Australia were shown to be reproductively isolated based on their different chromosome numbers and lack of shared alleles at five allozyme loci (Fig. 16.3). The long-footed potoroo is a distinct species that exists in very low numbers and is endangered. Morphologically similar velvet worms from the same log in the Blue Mountains inland from Sydney, Australia had fixed differences at 86% of 21 allozyme loci and were clearly different species that had diverged millions of years ago (Briscoe & Tait 1995).

#### Long-footed potoroo



#### Potoroo



**Fig. 16.3** Two sympatric potoroos (macropods) in southeastern Australia belong to separate species, as indicated by lack of gene exchange (Seebeck & Johnston 1980; Johnston *et al.* 1984). Between 1967 and 1978, four long-footed potoroos were collected in southeastern Australia that differed from the species known in that area in having longer hind feet and about twice the body size. The two forms have different chromosome numbers (24 chromosomes, versus 12 in females and 13 in males) and do not share alleles at 5 of 22 allozyme loci.



Long-footed potoroo

## Delineating allopatric species

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Allopatric populations that differ chromosomally are often distinctive species. However, the use of other genetic markers is less definitive, and requires calibration against genetic differentiation of other recognized species

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Establishing the taxonomic status of allopatric populations is often more difficult, as they typically have no opportunity for exchange of genetic material. The biological species concept would ideally require hybrid sterility, or markedly reduced survival to unequivocally distinguish species. Conversely, if the hybrids were fully viable and fertile through several generations, then the populations would belong to the same species. Such crosses are usually impractical, especially in threatened species.

Consequently, genetic markers are used to delineate allopatric species. Fixed chromosomal differences normally provide definitive evidence for distinct species status, as many chromosomal differences result in partial sterility in heterozygous individuals. For example, Chinese and Indian muntjac deer are morphologically similar, but are clearly distinct species as they have different chromosome numbers (Ryder & Fleischer [1996](#)). Data on rock wallabies and native rodents in Australia suggest that chromosomes

provide better predictions of reproductive isolation than do molecular markers (Eldridge & Close 1992; P. R. Baverstock *et al.* pers. comm.). Similarity of chromosomes in allopatric populations is not definitive evidence of conspecificity. Regrettably, chromosomal analyses are currently out of fashion for delineating taxonomic status, but deserve to be revisited.

Mitochondrial DNA is one of the most commonly used genetic markers to delineate taxa. However, as it is generally maternally inherited, mtDNA differentiation can be produced by lack of female dispersal. Further, patterns in different populations can be misleading as a result of selection or polymorphism in the ancestral species (Nei 1996; Box 16.3). Consequently, it is unwise to use mtDNA as the sole basis for delineating taxonomic status.

In the absence of crossing data, the most convincing delineations of species are based on the concordance of a wide array of information including morphology, breeding behaviour, chromosomes, nuclear markers and mtDNA. In the future, we envisage that molecular comparisons of loci involved in reproductive isolation will improve our delineation of species.

Classification based on molecular markers is more arbitrary than for sympatric species, as it is based on inferred reproductive isolation. In practice, two populations are considered to be different species if they are as genetically differentiated as are two well-recognized species in a related group. For example, Bornean and Sumatran orangutans differ in mtDNA, proteins and DNA fingerprints, and by a chromosomal inversion, as much as do other, well-known, distinct primate species. Consequently, it has been suggested that they should be classified as different species, as opposed to two sub-species (Box 16.2). In contrast, crosses between the two forms are viable and fertile in the  $F_1$  and  $F_2$ , suggesting conspecificity.

Genetic markers have been used to establish that newly discovered mammals in Vietnam and Laos are distinct from known species. For example, the saola or Vu Quang bovid has been identified as a distinct species based on morphology and DNA analyses (Whitfield 1998).

If differentiation between allopatric populations is much less than that between two well-recognized species in the same or related genera, then the populations are considered to belong to the same species. For example, the colonial pocket gopher population from Georgia, USA consisted of less than 100 individuals in the 1960s and was listed as an endangered species. Subsequent analyses based on morphology, allozymes, chromosomes and mtDNA revealed no consistent differences between this population and nearby populations of the common southeastern pocket gopher, so it does not warrant recognition as a separate species (Laerm *et al.* 1982). Similarly, the Cape Verde kite is not a separate species from the black kites (Box 1.1).



Pocket gopher

## Genetic distance

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Nei's genetic distance is the most commonly used measure of genetic differentiation among populations and species

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A measure of genetic differentiation or **genetic distance** is required to aid in determining the status of allopatric populations as populations, sub-species or distinct species. We can then compare this distance with that among 'good' species in related groups. The most commonly used measure is Nei's genetic distance  $D_N$  (Nei 1987). We first define Nei's index of genetic similarity  $I_N$

$$I_N = \frac{\sum_{i=1}^m (p_{ix} p_{iy})}{\left[ \left( \sum_{i=1}^m p_{ix}^2 \right) \left( \sum_{i=1}^m p_{iy}^2 \right) \right]^{1/2}} \quad (16.1)$$

and then take natural logarithms to obtain Nei's genetic distance

$$D_N = -\ln(I_N) \quad (16.2)$$

where  $p_{ix}$  is the frequency of allele  $i$  in population (or species)  $x$ ,  $p_{iy}$  is the frequency of allele  $i$  in population (or species)  $y$ ,  $m$  is the number of alleles at the locus and  $\ln$  is the natural logarithm.



Red-cockaded woodpeckers

When allele frequencies are similar in two populations ( $p_{ix} = p_{iy}$ ), the genetic similarity approaches one, and the genetic distance approaches zero. Conversely, when the two populations share no alleles, the index of genetic similarity is zero and the genetic distance is infinity. Example 16.1 illustrates

calculation of genetic distance from an allozyme locus in endangered red-cockaded woodpeckers. Estimates should be based on numerous loci to provide reliable genetic distances (Nei & Takezaki 1994). The method for combining information for different loci is given by Nei (1987).

### Example 16.1 Calculation of Nei's genetic distance

Three populations of endangered red-cockaded woodpeckers have the following frequencies at the lactate dehydrogenase (LDH) locus (from Stangel *et al.* 1992).

Ldh allele	Frequencies		
	Vernon	Apalachicola	Gameland
B	0.023	0.019	0.981
C	0.977	0.885	0.019
D	0.000	0.096	0.000

To calculate the genetic distance we need to compute the squares of the allele frequencies and the products of their frequencies between populations.

For the Vernon population the sum of the squared frequencies is

$$\Sigma p_{ix}^2 = 0.023^2 + 0.977^2 + 0.000^2 = 0.955$$

and for Apalachicola

$$\Sigma p_{iy}^2 = 0.019^2 + 0.885^2 + 0.096^2 = 0.793$$

The numerator is the sum of the cross products, as follows:

$$\Sigma(p_{ix} p_{iy}) = 0.023 \times 0.019 + 0.977 \times 0.885 + 0 \times 0.096 = 0.865$$

Consequently, Nei's genetic similarity for the Vernon and Apalachicola comparison is

$$I_N = \frac{\sum(p_{ix} p_{iy})}{[(\sum p_{ix}^2)(\sum p_{iy}^2)]^{1/2}} = \frac{0.865}{(0.955 \times 0.793)^{1/2}} = \frac{0.865}{0.870} = 0.994$$

and the genetic distance is

$$D_N = -\ln(I_N) = -\ln(0.994) = 0.006$$

Consequently, the genetic distance between the Vernon and Apalachicola populations of the woodpeckers is only 0.006, i.e. the populations are genetically very similar.

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## How large are genetic distances for ‘good’ species?

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Genetic distances generally increase with level of reproductive isolation, but the relationship is very ‘noisy’

The extent of reproductive isolation among populations is correlated with their genetic differentiation, and genetic distances generally increase as we progress up the taxonomic hierarchy from populations within species, to species, etc. (Coyne & Orr [2004](#)). For example, average allozyme genetic distances in fruit flies of the *Drosophila willistoni* species complex increase from geographically isolated populations (mean  $D = 0.03$ ), to sub-species ( $D = 0.23$ ) to distinct species ( $D = 1.21$ ). However, the relationship is very approximate and noisy. For example, sub-species of lizards show greater genetic distances than species of macaques, gophers and birds and the human–chimpanzee family difference is less than many species differences in other groups (Nei [1987](#)). Designations of taxonomic status based on genetic distance for allozyme loci are imprecise, and a similar conclusion applies to

mtDNA genetic distances in vertebrates (Johns & Avise [1998](#)).

## Constructing phylogenetic trees

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Information from genetic or morphological markers can be used to construct phylogenetic trees

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We are often interested in evolutionary relationships among populations and species (see [Chapters 17](#) and [21](#)). For example, we may wish to identify the most closely related population or sub-species to use in crossing programs to recover threatened species. If the true relationships among the six sub-species of seaside sparrow had been known, the last of the dusky sub-species would have been crossed, probably successfully, to a related Atlantic coast sub-species rather than to a less related Gulf coast form. Phylogenetic trees have also been recommended for use in measuring phylogenetic distinctiveness, as a guide to allocating conservation resources (Crozier *et al.* [2005](#)).

Phylogenetic trees reflecting evolutionary relationships among species (or populations) can be constructed using genetic data (Avise [2000](#); Nei & Kumar [2000](#)). Example 16.2 provides a simple illustration of tree building based on mtDNA sequence data for the Norfolk Island boobook owl and its nearest presumed relatives.

A large number of statistical methods are now available for deriving trees from molecular markers or from morphology, including distance matrix methods (UPGMA), maximum parsimony, maximum likelihood and Bayesian methods (Nei & Kumar [2000](#); Felsenstein [2004](#); Hall [2004a](#)).



Maximum parsimony simply builds a tree based upon the minimum number of changes, no matter what those changes are. More complex models allow for differences between the rates of transition and transversion mutations (the former usually being more common), between base frequencies, etc., and can allow for evolutionary rate heterogeneity across sites (Whelan *et al.* [2001](#)).

The above methods generally yield reliable trees and are concordant if there is sufficient information, i.e. numbers of loci, number of nucleotides or amino acids or number of morphological characters, or preferably a combination of these (Nei [1996](#)). Major software packages for constructing phylogenetic trees are listed at the end of the chapter. Readers are referred to Felsenstein ([2004](#)), Hall ([2004b](#)) and Kolaczkowski & Thornton ([2004](#)) for further details on the methods.

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### **Example 16.2 Building a phylogenetic tree from DNA sequence data**

The Norfolk Island (NI) boobook owl declined to a single individual, and the best recovery option was to cross it to its most closely related sub-species. Crosses of the remaining female to males from a related New Zealand (NZ) sub-species have yielded about 16 F<sub>1</sub> offspring. Subsequent analyses of mtDNA sequences (298 bases of the cytochrome *b* locus) confirmed that the sub-species chosen for crossing was the most closely related and conformed with morphological data (Norman *et al.* [1998](#)).

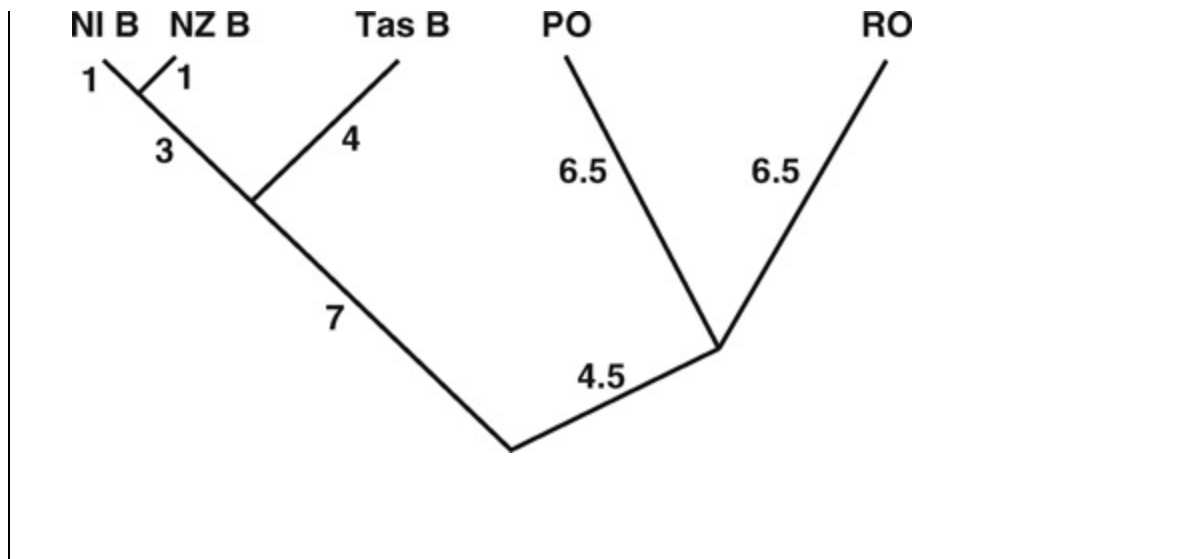


Norfolk Island boobook owl

Comparison	mtDNA base pair differences
Norfolk Island boobook – NZ boobook	2
Norfolk Island boobook – Tas boobook	8
NZ boobook – Tas boobook	8
Powerful owl – rufous owl	13
Norfolk Island boobook – powerful owl	21
Norfolk Island boobook – rufous owl	23

The UPGMA method works by placing the nearest relatives closest together, and building a tree where lengths of the segments are proportional to the number of base differences between taxa. Thus, the branch lengths to the node below NI boobook and NZ boobook are 1 each (total of 2 differences between them). These differ from Tas boobook by 8 bases, so we attribute 4 to the segment from Tas boobook to the node below it, and 4 from the node to NI boobook and NZ boobook. As one base difference has already been attributed to the distance to the NI boobook–NZ boobook node, 3 are attributed to the section from their node to the joint node with Tas boobook. Half of the difference of 13 between powerful owl and rufous owl (6.5) is attributed to each path to their node. Finally the NI boobook–powerful owl and NI boobook–rufous owl average 22 bases different, so we attribute half of this (11) to each path to the lowest node.

On the left-hand side we have already attributed 4, leaving 7 for the section to the lowest node. On the right-hand side, 6.5 has been allocated to the path to the first node, leaving 4.5 to allocated to the path from this node to the lowest node.



Trees based on genetic markers may not accurately reflect evolutionary relationships if rates of evolution are not constant in different lineages, markers are not neutral, or the foundation population is polymorphic. The last issue can be overcome by using many independently inherited markers

**Box 16.3** uses examples to illustrate the effects of polymorphism in the founder population, selection and unequal rates of evolution on inferred phylogenetic trees. For example, the mtDNA tree for primates disagrees with information from a wide array of morphological, behavioural, chromosomal and other genetic evidence, probably as a consequence of selection. When selection occurs or mutation rates are unequal in different lineages (B), rates of evolution are not equal in different lineages, the lengths of branches are distorted and the inferred tree may differ from the true tree. The consensus phylogeny in primates, based on several nuclear loci, is concordant with the other evidence (Herbert *et al.* 1999).

When the starting population is polymorphic (C), as will frequently be the case, fixation of different initial sequences in different lineages (termed **lineage sorting**) may lead to incorrect inferred phylogenies. This situation can be resolved if data are available from many independent loci; Nei & Takezaki (1994) recommended about 30 polymorphic allozyme loci or 20 microsatellite loci. Transposable element insertions provide highly informative markers, as integration of a particular element at a specific location in the genome is irreversible and of known polarity, whilst repeated substitutions at a DNA site can reverse a particular change, leading to apparent similarity (**homoplasy**) (Takahashi *et al.* 2001; Kriegs *et al.* 2006).

## Outbreeding depression

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Outbreeding depression is the reduction in reproductive fitness that can occur from crossing of diverged populations

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Crossing of genetically differentiated populations or higher taxa creates the risk that hybrid offspring in the first and subsequent generations will suffer reduced reproductive fitness.

Outbreeding depression may be expected in crosses between different sub-species or species

Many well-defined sub-species have diverged to the point where crosses among them result in reduced fitness. Crosses between Bengal and Siberian tigers would not be expected to produce offspring fit for either environment. A major reason for resolving taxonomic uncertainties is to avoid such crosses.



Tiger

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The risk of outbreeding depression is elevated when populations have adapted to different habitats and increases with generations of differential adaptation

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As the main factor driving reproductive isolation and speciation is differential natural selection, adaptation to different environments is the main risk factor for outbreeding depression. This risk increases with generations of adaptation to different environments (Rice & Hostert 1993; R. Frankham *et al.* unpublished theory and analyses). Conversely, populations in similar environments for a limited number of generations have low risk of outbreeding depression. For example, crosses among fruit fly populations maintained for up to 170 generations in the same environment show no outbreeding depression. Conversely, some populations maintained in different environments developed modest outbreeding depression. In general, isolated populations have limited risks from outcrossing if they share similar environments, have the same karyotypes, previously exchanged genes and have long generation intervals (e.g. large mammals). Conversely, the risks are considerable if populations have short generation intervals (e.g. invertebrates and annual plants), have been long isolated and are in different habitats.

## **Extent of outbreeding depression in animals and plants**

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Outbreeding depression in crosses between populations within species has been documented in relatively few animal cases, but may be more common in plants

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The extent and significance of outbreeding depression is a matter of controversy. Cases of outbreeding depression in mammals and birds are few, apart from those where the taxonomy has not been adequately resolved (R. Frankham *et al.* unpublished data). The most widely quoted, but anecdotal, example is ibex in the Tatra Mountains of Slovakia (Turcek 1951). The population went extinct following introduction of desert-adapted animals of a different sub-species from Turkey and Sinai to the European population. Maladapted hybrids mated in early autumn and, fatally, gave birth in February, the coldest month.

The most comprehensive evidence comes from a meta-analysis of crosses between fish populations within species. Crosses showed diverse effects on fitness, but on average tended to be beneficial (McClelland & Naish 2007). Further, species crosses are almost equally likely to be beneficial as deleterious (Arnold 1997). Outbreeding depression has been detected for some traits in experimental crosses between sub-species of mice, but is less than the beneficial effects of crossing (heterosis) (Lacy 1998). The endangered Arabian oryx is suffering simultaneously from both inbreeding depression and outbreeding depression, but there are chromosomal differences segregating in this population that may explain the outbreeding depression (Benirschke & Kumamoto 1991; Marshall & Spalton 2000).

In several cases where outbreeding depression has been detected in animals, the presumed taxonomy proved to be erroneous (R. Frankham *et al.* unpublished data). For example, different populations of owl monkeys and

dik-diks that came from different localities and had different chromosome numbers, produced sterile offspring when crossed, and were probably different, undescribed species (Templeton [1986](#)). Further, two populations of corroboree frogs in Australia that showed modest outbreeding depression have been reclassified as distinct species (Osborne *et al.* [1996](#)).

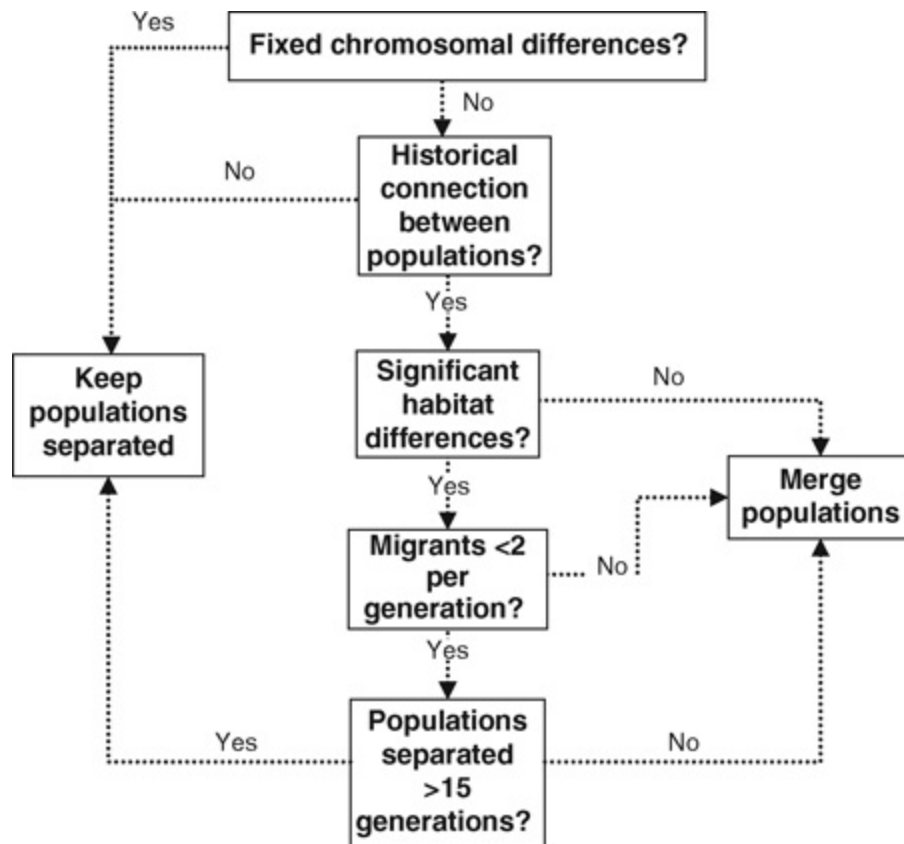


Corroboree frog

Most evidence for outbreeding depression comes from plants and animals with limited dispersal characteristics and adaptation to different environments (Waser [1993](#); Dudash & Fenster [2000](#)). For example, Dudash & Fenster ([2000](#)) found simultaneous inbreeding and outbreeding depression in an outbreeding legume in the USA, but outbreeding depression was only notable in crosses between populations from locations 2000 km apart. Outbreeding depression has been clearly documented in an intertidal copepod that shows marked genetic differentiation, and limited dispersal, over relatively short geographic distances (Burton *et al.* [1999](#)). However, these populations have been isolated for up to 16.6 million years (see Edmands *et al.* [2005](#)). Quantitative estimates of the frequency, magnitude and distribution of outbreeding depression for a broader array of taxa are required without delay.

Currently, great caution is expressed about mixing populations. For example, doubts have been expressed about the wisdom of crossing different populations of gray wolves, even though they clearly belong to the same species (Shields [1984](#)). Many population geneticists consider that concerns over outbreeding depression, for species whose taxonomy is clearly understood, are being overemphasized. Outbreeding depression is frequently mentioned, but the benefits of crossing are understated. [Figure 16.4](#) presents a flowchart of questions to ask to assess the risk of outbreeding depression

and the practical decision that results.



**Fig. 16.4** Flowchart of questions to ask to assess the risk of outbreeding depression and the practical decision that results (R. Frankham *et al.*, unpublished theory and analyses).

Even if crosses of populations result in immediate outbreeding depression, natural selection will usually bring about rapid recovery and, often, higher eventual fitness

Even where outbreeding depression occurs following crossing of two partially inbred and differentiated populations, it will not be a long-term



phenomenon. Unless the  $F_1$  hybrid individuals are of very low fitness, natural selection will act upon the extensive genetic variation in the hybrid population, resulting in improved fitness and adaptation to its environment, as observed in several cases (Lewontin & Birch 1966; Rieseberg *et al.* 1996; Carney *et al.* 2000; Erickson & Fenster 2006).

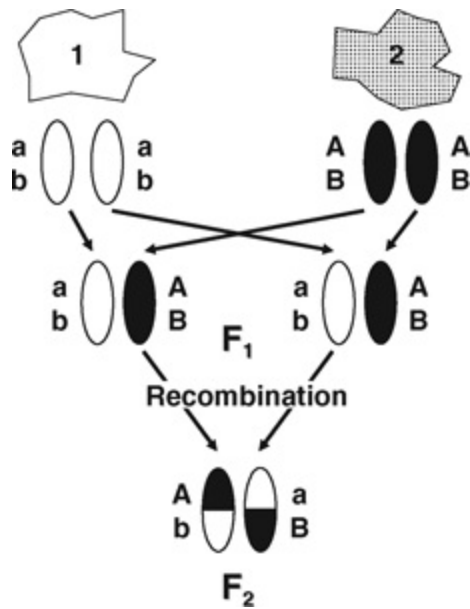
## Genetic basis of outbreeding depression

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Outbreeding depression may arise from combining alleles causing adaptation to different environments in populations, so that the hybrid populations are not well adapted to either environment

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Outbreeding depression may arise due to two different mechanisms. First, populations adapted to different environmental conditions will perform poorly in the non-native environment (Fig. 5.7) and hybrid populations will contain genotypic combinations of alleles that may be less suited to either environment (Fig. 16.5). The  $F_1$  hybrids between the European and Middle Eastern ibex (above) calved at a time intermediate between that of the two parent populations. However, this was in mid-winter and all offspring died.



**Fig. 16.5** Genetic mechanisms of outbreeding depression. The diagram shows how genotypes and sets of alleles at two loci (coadapted gene complexes) adapted to different local environments (shaded vs. unshaded) could reduce fitness of  $F_1$  and  $F_2$  hybrids (after Templeton & Read 1984). Here genotypes  $aa\ bb$  and  $AA\ BB$  are adapted to environments 1 and 2, respectively. Furthermore, the two alleles  $a/b$  and  $A/B$  form coadapted gene complexes that have high fitness only when containing alleles from the same source population. Fitness reductions in the  $F_1$  can result when alleles from different populations form genotypes maladapted to the environment in which the  $F_1$  reside. For example,  $aa$  and  $AA$  are adapted to their original environments, while the  $F_1$  genotype  $Aa$  is adapted to neither. Fitness can be further reduced in the  $F_2$  when segregation and recombination break up the coadapted gene complexes. After recombination, allele  $a$  is with  $B$ , and  $A$  is with  $b$ .

Populations that have adapted by utilizing different sets of interacting alleles (coadapted gene complexes) may show outbreeding depression as alleles are deleterious when recombined

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A second more complex type of outbreeding depression occurs when different populations have evolved different **coadapted gene complexes**. Coadapted gene complexes are groups of alleles at multiple loci whose fitness depends on interactions among loci (epistatic interactions). This can even evolve in populations adapted to similar environments. For example, one population may evolve genotype aabb and another AABB (Fig. 16.5) because alleles a and b are beneficial together, as are A and B. When they are crossed, segregation and recombination among the genomes in hybrid populations produces new allelic combinations (e.g. AAbb and aaBB genotypes), that are deleterious. Outbreeding depression resulting from disruption of coadapted gene complexes has been shown in a copepod, mice and a legume *Chamaecrista fasciculata* (Lacy 1998; Burton *et al.* 1999; Dudash & Fenster 2000). However, evidence generally does not support the importance of this mechanism. Crosses between populations of fruit flies adapted to the same environment rarely show outbreeding depression (Rice & Hostert 1993).

Techniques for distinguishing outbreeding depression due to disruption of coadapted gene complexes from inbreeding depression are given by Lynch (1991) and Marshall & Spalton (2000).

## Defining management units within species

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Populations within species may be sufficiently differentiated in adaptive characteristics, or genetic composition, to require separate management

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Populations within species that are on the path to speciation may justify separate management. The desirability of separate management depends on the balance between the cost of keeping two (or more) populations versus one, and the risks of outbreeding depression, or benefits (higher genetic diversity and fitness) accruing from hybridizing the populations. Below we outline the concept of **evolutionarily significant units**, together with another proposal to define such units based on genetic and ecological exchangeability.

## Evolutionarily significant units (ESU)

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An evolutionarily significant unit (ESU) is a population that has a high priority for separate conservation

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Many conservation biologists believe that genetically differentiated populations within species require separate genetic management and should only be merged as a last resort (see Moritz [1995](#)). These populations are referred to as **evolutionarily significant units**. Initially the concept was applied to populations with reproductive and historical isolation and adaptive distinctiveness from other populations within the species (Crandall *et al.* [2000](#)). Moritz ([1995](#)) proposed that genetic markers be used to define management units within species. If mtDNA shows no overlap between populations (**reciprocal monophyly**), and nuclear loci show significant divergence of allele frequencies, then they should be defined as separate ESUs and managed separately. In broad terms this often means that well-defined sub-species are the unit of management.

While many ESUs have been defined in threatened species, the concept has been criticized. In particular, ESUs defined solely using neutral molecular

genetic markers ignore adaptive differences (Crandall *et al.* 2000). ESUs are unlikely to be detected within species with high gene flow, even though populations may have adaptive differences (Box 7.3) and perhaps warrant separate management. Conversely, in taxa with low gene flow, populations that have differentiated by genetic drift may be designated as separate ESUs, even though they may not be adaptively distinct – in this case they may benefit from gene flow.

## Defining management units on the basis of exchangeability

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Management units can be defined using ecological and genetic exchangeability

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Crandall *et al.* (2000) proposed that management units within species be based upon whether populations are ecologically or genetically replaceable by one another (**exchangeable**). This proposal is based on whether there is adaptive differentiation, whether there is gene flow and whether differentiation is historical, or recent. In practice, the populations are given \* (reject exchangeability) or 0 (accept) classifications in each of four cells, representing recent and historical genetic and ecological exchangeability, resulting in 16 categories of divergence between two populations (Table 16.1). In general, the more \* scores the greater the differentiation.

**Table 16.1 Defining management units within species on the basis of genetic and ecological exchangeability and recommended management for different categories (Crandall *et al.* 2000). Categories of population distinctiveness are based on rejection (\*) or failure to reject (0) the null hypothesis of genetic and ecological exchangeability for both recent and historical time frames. As the number representing relative strength of the evidence increases, so does the evidence for significant population**

## differentiation

Relative strength of evidence (indicated by number)	Evidence of adaptive distinctiveness	Recommended management action
8	$\frac{**}{**}$	Treat as separate species
7	$\frac{**}{0/0}$ or $\frac{**}{**/0}$	Treat as separate species
6	$\frac{0/0}{**}$	Treat as distinct populations (recent admixture, loss of genetic distinctiveness)
5	$\frac{**0}{**}$	Natural convergence on ecological exchangeability – treat as single population
4	(a) (b) (c) $\frac{**}{0/0}$ $\frac{0/0}{0/0}$ $\frac{0/0}{**}$	Anthropogenic convergence – treat as distinct populations. (a) and (b) Recent ecological distinction, so treat as distinct populations. (c) Allow gene flow consistent with current population structure
3	$\frac{0/0}{0/0}$	Allow gene flow consistent with current population structure; treat as distinct populations
2	$\frac{**0}{**/0}$	Allow gene flow consistent with current population structure; treat as a single population
1	$\frac{**0}{0/0}$ $\frac{0/0}{0/0}$ $\frac{0/0}{**}$ $\frac{**0}{0/0}$ $\frac{0/0}{**}$ $\frac{0/0}{0/0}$	Treat as single populations; if exchangeability is due to anthropogenic effects, restore to historical condition; if natural, allow gene flow

### $H_0$ exchangeability

Time frame	Genetic	Ecological
Recent		
Historical		

Genetic exchangeability is concerned with the limits to spread of new genetic variation through gene flow. Exchangeability is rejected (\*) when there is evidence of restricted gene flow between populations, while it is accepted when there is evidence of ample gene flow (0). Evidence for gene flow is ideally based on multiple nuclear loci (allozymes, microsatellites, etc.).

Ecological exchangeability is rejected where there is evidence for population differentiation due to natural selection, or genetic drift. Evidence

can be based on difference in life history traits, morphology, habitat and loci under selection. Such differences should, ideally, be demonstrably heritable, based on measurements of fitness traits in reciprocal transplants or common garden experiments over two generations (Rader *et al.* 2005). This primarily reflects adaptive differentiation.

The recent and historical time frames are designed to distinguish natural evolutionary processes of limited gene flow from recent population isolation. Further, they distinguish secondary contact from long-term gene flow.

Recommended management actions are given for each of the 16 categories, collected into eight management groups in Table 16.1. Example 16.3 illustrates the application of the methodology to black rhinoceros, Cryan's buckmoths and puritan tiger beetles. This methodology provides a logical means for delineating populations that justify separate management, without having an excessive number of management units that do not show adaptive differentiation. By contrast, the term ESU has been applied to every category in Table 16.1, leading to a very large number of management units, some with doubtful justification. The authors claim that this system deals more adequately with many cases where the ESU process yielded outcomes of dubious validity. However, the required data on adaptive differentiation are often not available.

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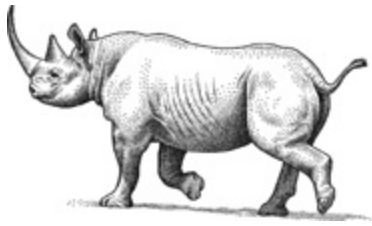
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### **Example 16.3 Assignment of black rhinoceros, Cryan's buckmoth and puritan tiger beetles to the categories defined by Crandall et al. (2000)**

#### **Black rhinoceros**

For the black rhinoceros in Africa, there are insufficient grounds to reject either genetic exchangeability or ecological exchangeability. Populations show gene flow and their habitats are similar. Consequently, it is categorized as category 1( $\frac{010}{010}$ ), leading to the recommendation that the species be managed as a single population. Conversely, mtDNA data have

been used to argue for two sub-species with separate management (O'Ryan *et al.* 1994).



Black rhinoceros

### **Cryan's buckmoth**

For Cryan's buckmoth, Legge *et al.* (1996) found no evidence to reject genetic exchangeability based on either mtDNA or allozymes (there was adequate gene flow), yet there was evidence to reject recent, but not historical ecological exchangeability among populations ( $\frac{0|*}{0|0}$ ) (category 3). Both the authors and Crandall *et al.* (2000) argued for the adaptive significance of the ecological differentiation and thus for the recognition of separate management units, although gene flow consistent with current population structure is allowable.

### **Puritan tiger beetle**

Puritan tiger beetles from Connecticut River and Chesapeake Bay, USA are not genetically exchangeable, based on mtDNA (low gene flow and significant differentiation). Further they are not ecologically exchangeable based on habitat parameters. Thus they were classified as category 7 ( $\frac{*|*}{*|0}$ ), indicating strong adaptive differentiation and the recommendation to manage the two populations as separate units for conservation purposes. Populations on the east and west coasts of Chesapeake Bay were genetically and ecologically exchangeable( $\frac{0|0}{0|0}$ ).

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## **Summary**

1. Correct diagnosis of taxonomic status of populations is critical for



conservation purposes so that undiagnosed species are not left to become extinct, undiagnosed species are not hybridized with deleterious consequences, or resources are not wasted on populations mistakenly identified as endangered species, or on hybrid populations that are not distinct species.

2. There is no universally accepted definition of species.
3. The biological species concept has been highly influential in population and conservation biology. It defines species as groups of actually, or potentially, interbreeding individuals that are isolated from other such groups.
4. According to this concept, genetic markers can be used to diagnose species definitively among sympatric populations, as different species will not be exchanging genes.
5. For allopatric populations, definitive diagnosis of species requires information on the fertility and viability of crosses between populations. Major chromosomal differences also lead to definitive classification. Molecular markers also assist in diagnosing taxonomic status, but this is more subjective and must be calibrated against genetic differentiation of known 'good' species in related groups of organisms.
6. Outbreeding depression is the reduction in fitness that can occur when some populations are crossed. It is more likely when there are many generations of differential adaptation to diverse environments, and restricted gene flow.
7. Populations within a species may justify management as separate units if they show adaptive genetic differentiation. Delineation of such populations requires information on their genetic and ecological exchangeability – if they show significant differentiation in both, they should be managed separately.

### **Further reading**

Catalogue of Life: A comprehensive catalogue that plans to include all known species of organisms on Earth by 2011. [www.catalogueoflife.org/](http://www.catalogueoflife.org/)

Consortium for the Barcode of Life: Universal barcode library to which comparisons of unidentified taxa can be made. [www.barcoding.si.edu](http://www.barcoding.si.edu)

Coyne & Orr (2004) *Speciation*. Authoritative and scholarly review of speciation.

Crandall *et al.* (2000) Defines method for using genetic and ecological exchangeability between populations to designate populations deserving separate management.

Dasmahapatra & Mallet (2006) Brief review of advantages and limitations of DNA barcoding.

Felsenstein (2004) *Inferring Phylogenies*. Book on building phylogenetic trees from one of the major figures in this field.

Gavrilets (2004) *Fitness Landscapes and the Origin of Species*. Book on speciation with a theoretical emphasis.

Hall (2004a) *Phylogenetics Made Easy*. A clearly written guide to building phylogenetic trees from molecular data.

Rieseberg & Willis (2007) Authoritative recent review on plant speciation.

## Software

BEAST: Bayesian Evolutionary Analysis Sampling Trees software for inferring phylogenetic trees and testing evolutionary hypotheses from molecular data (Drummond & Rambaut 2007). [http://beast.bio.ed.ac.uk/Main\\_Page/](http://beast.bio.ed.ac.uk/Main_Page/)

MrBayes 3: Free package for estimating trees by Bayesian methods (Ronquist & Huelsenback 2003). <http://morphbank.ebc.uu.se/mrbayes/>

PAUP: Commercial package that implements a wide variety of methods for phylogenetic inference. [www.sinauer.com/titles/frswofford.html](http://www.sinauer.com/titles/frswofford.html)

PHYLIP: Felsenstein's very informative website with free programs for phylogenetic inference using a wide range of methods.

[www.evolution.genetics.washington.edu/phylip.html](http://www.evolution.genetics.washington.edu/phylip.html)

TreeBASE: Phylogenetic database of DNA sequences among species and phylogenies derived from them. [www.treebase.org/](http://www.treebase.org/)

## Problems

**16.1** Taxonomic uncertainties. Why are taxonomic uncertainties of conservation concern?

**16.2** Taxonomy. What is meant by sympatric?

**16.3** Taxonomic uncertainties. Sympatric populations show the following characteristics. Are they the same or different species? Why?

- (a) Diploid chromosome number 20 in population A and 22 in population B.
- (b) Allozyme genotypes at the Adh locus of SS in one population, and FF in the other (S and F represent different electrophoretic mobilities).
- (c) Microsatellite genotypes 11, 12 and 22 in one population and 33, 34 and 44 in another (1, 2, 3 and 4 are microsatellite alleles with different sizes).
- (d) Microsatellite genotypes 11, 12 and 22 in one population, and 11 in the other.

**16.4** Taxonomy. What is meant by allopatric?

**16.5** Taxonomic uncertainties. Allopatric populations show the following characteristics. Are they the same or different species? Why?

- (a) Giant redwood trees in California have 66 chromosomes in all individuals, while a morphologically related plant population in China has 22 chromosomes in every individual (Lewis 1980).
- (b) Tigers have an extensive distribution on the Asian mainland, while the Sumatran population is on an island isolated by

rising water levels 6000–10 000 years ago. Separation based on morphology is minimal. Crosses between tiger sub-species are fertile. For mtDNA sequences in the cytochrome *b* locus, 3 bases out of 1140 show fixed differences between mainland and Sumatran tigers (Cracraft *et al.* 1998).

- (c) Two populations of velvet worm, one from the Blue Mountains near Sydney, Australia and the other from the Border Range about 1000 km away, show fixed differences at 70% of 20 loci surveyed (D. A. Briscoe & N. N. Tait pers. comm.).

**16.6** Genetic distance. Calculate Nei's genetic distance for two populations *x* and *y* with the following frequencies for alleles at a single locus.

	Allele		
	1	2	3
(a) Population <i>x</i>	0.1	0.2	0.7
Population <i>y</i>	0.1	0.2	0.7
(b) Population <i>x</i>	0.5	0.5	0.0
Population <i>y</i>	0.0	0.0	1.0
(c) Population <i>x</i>	0.5	0.5	0.0
Population <i>y</i>	0	0.7	0.3

**16.7** Genetic distance. Calculate Nei's genetic distances using the data in Example 16.1 between the Vernon and Gameland populations.

**16.8** Management units. Use the exchangeability method of Crandall *et al.* (2000) to categorize three coho salmon populations. Populations A and B differ significantly from C in microsatellite frequencies and in heritable characters likely to be of ecological significance (morphology, swimming ability and age at maturation). How would you recommend the three populations be managed?

**16.9** Outbreeding depression. What is outbreeding depression? Why is it of conservation concern? What are the major factors affecting the risk of its occurrence?

## Practical exercise: Building a phylogenetic tree

Use DNA sequence data to build a phylogenetic tree using a computer software package, such as PAUP or PHYLIP. Suitable DNA sequence data on primates can be found in Hayasaka *et al.* ([1988](#)), while sequences for other species can be found in GENBANK or TREEBASE. Determine whether you obtain the same tree using different methods.

## Chapter 17 Genetic management of wild populations

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Genetic management of endangered species *in situ* involves the recovery of small inbred populations, management of fragmented populations, and minimizing the deleterious impacts of harvesting

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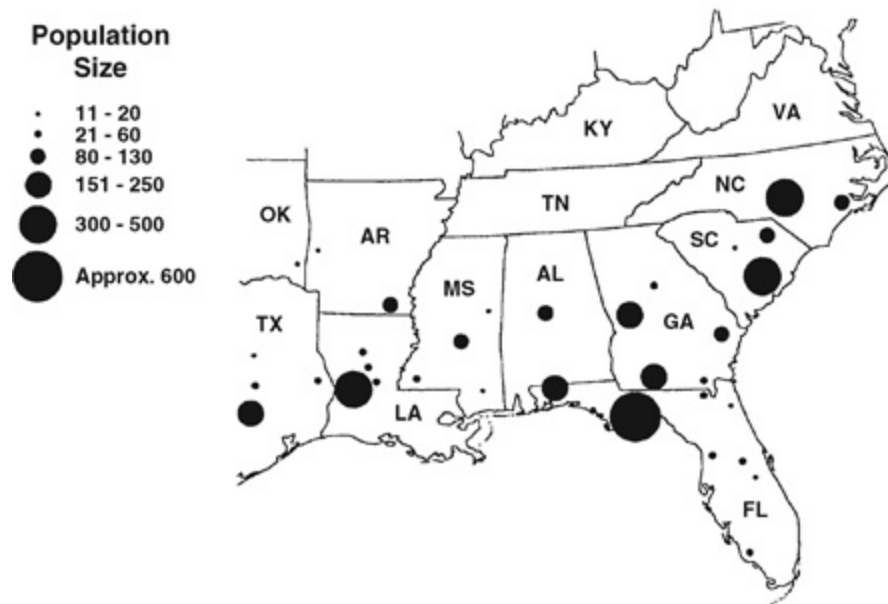
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### **Terms**

Clones, corridor, genetic erosion, genetic rescue, gynodioecious, translocation

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The endangered red-cockaded woodpecker and its current fragmented distribution in southeastern USA (from James 1995)

## Genetic issues in wild populations

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Genetic management of threatened populations in situ is in its infancy

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There has been only limited application of genetics in the practical management of threatened taxa in natural habitats. Genetic issues are important in wild populations but, unfortunately, this importance is rarely appreciated. We have already referred to many of the known examples of genetic management of wild populations and a number are presented in more detail here.

To summarize what we have discussed previously, the key genetic contributions to conservation biology are:

- resolving taxonomic uncertainties so that managers can be confident of the status of, and relationships among, the populations they strive to maintain ([Chapter 16](#))
- delineating any distinct management units within species ([Chapter 16](#))
- detecting declines in genetic diversity ([Chapter 11](#))
- developing theory to describe past, and predict future, changes in genetic variation and inbreeding. All such theories show that genetic impacts depend on  $N_e$  ([Chapters 11–14](#))
- recognizing that the effective sizes of populations are frequently about an order of magnitude lower than their census sizes ([Chapter 11](#))
- recognizing that loss of genetic diversity for reproductive fitness reduces the capacity of populations to evolve in response to environmental change ([Chapters 8, 11 and 15](#))
- recognizing that inbreeding depression is an expected result of extensive inbreeding in almost all cases ([Chapter 13](#))



- recognizing that potential inbreeding depression may be inferred from its correlation with reduction in genetic variation
- recognizing that degree of fragmentation and rates of gene flow can be inferred from the distribution of genetic markers within and among populations.

Several of these issues are illustrated by the example of the Florida panther ([Box 17.1](#)).

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**Box 17.1 Identifying genetic problems in the Florida panther and genetic management to alleviate them (Roelke et al. [1993](#); Culver et al. [2000](#); Land & Lacy [2000](#); Mansfield & Land [2002](#); Gross [2005](#); Stokstad [2005](#); Pimm et al. [2006](#); Culver et al. [2008](#))**

The endangered Florida panther was restricted to a small relict population that went through a bottleneck of ~6 individuals for two generations and ~40 for a longer period in southern Florida, primarily in the Big Cypress and adjoining Everglades National Park ecosystems. Prior to European settlement, they ranged across the entire southeast of the United States, and other sub-species were spread throughout North and South America. Since 1973, the main causes of deaths have been road kills, illegal hunting or injuries. A population viability analysis in 1989 predicted a high probability of extinction within a short time, unless remedial actions were taken (Seal & Lacy [1989](#)). A more recent assessment is more optimistic, but the validity of the input data has been challenged (Gross [2005](#)).



Florida panther

Analyses using allozymes, morphology and mtDNA revealed that a portion of the population had received genetic input (introgression) from a South America puma sub-species between 1956 and 1966. Hybrid animals are located in areas away from most 'authentic' animals.

The authentic Florida panther population had very low levels of allozyme, DNA fingerprint, microsatellite and mtDNA genetic diversity compared to the hybrids, other puma populations and felids generally.

Sub-species	Allozymes $H_e$ (%) (range)	DNA	
		fingerprint $H_e$ (%)	Microsatellites $H_e$ (%)
Florida (authentic)	1.8	10.4	14.7
Florida (introgressed)	1.8	29.7	—
Florida (museum)	—	—	31.1
Western US	4.3 (2.0–6.7)	46.9	34.8
Other felids	3–8	—	—
Domestic cat	—	44.0	—

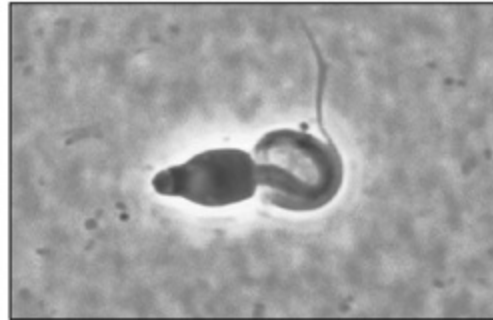
Authentic Florida panthers also displayed evidence of inbreeding depression, including morphological abnormalities (kinked tails), cardiac defects and very poor semen quality (see figure below; J. Howard & B. Pokazhhenthi). About half of 'pure' males had at least one undescended

testis and the incidence increased with time. Florida panthers also suffered a high prevalence of infectious disease.

Normal spermatozoon



Spermatozoon with an abnormal acrosome and tightly coiled tail



How could the authentic Florida panther be recovered? The first priority was to increase population size through protecting and improving additional habitat and decreasing existing threats. Construction of culverts under highways significantly reduced highway mortality. Since these panthers display both the genetic and physical hallmarks of inbreeding depression, their fitness was increased by immigration from the nearest sub-species from Texas, a population that was contiguous with the Florida population, and probably had historical gene exchange, before its decline. Outbreeding depression was unlikely as there was no evidence for it, either in the hybrid Florida animals or in mixed populations of captive pumas. Subsequent analyses revealed that all North American panthers/cougars are very similar for molecular markers.

Eight pumas from Texas were introduced in 1995, but most of the genetic inputs came from two or three of them. By 2004, 54 hybrid kittens had been produced, including some second generation and backcross offspring from hybrids. Hybrid offspring generally lacked kinked tails and cryptorchidism and appeared to be more robust than authentic Florida panthers. Hybrid kittens had about a three times higher chance of becoming adults than purebreds. Hybrid adult females survived better than purebred females, but there was no obvious difference between the males. Number and size of litters did not differ between purebred and hybrid females. Following the genetic rescue, panthers are expanding the known range of habitats that they occupy and use.

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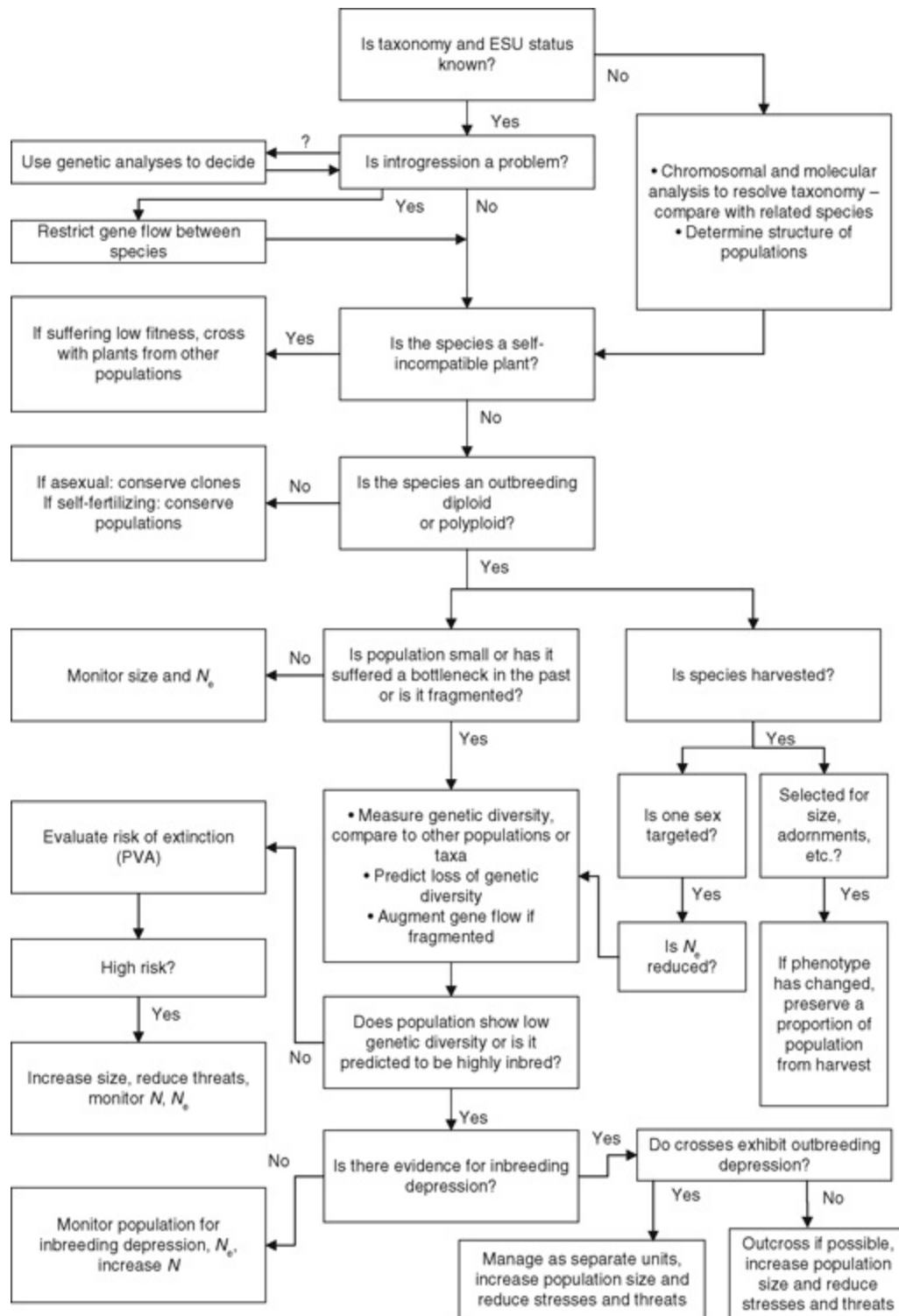
The goals of genetic management of wild populations are to maintain fitness by minimizing inbreeding and to ensure that there is adequate genetic diversity to allow adaptive evolution to environmental change

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By distilling the points above, we come to the conclusion that genetic management in the wild should have two main goals, to maintain reproductive fitness and to conserve genetic diversity for species and populations to adapt to environmental change.

This chapter follows approximately the sequence of genetic management actions for wild populations, as shown in the flowchart ([Fig. 17.1](#)). As in all conservation, before we can devise a management plan we must know what we are conserving and have clear conservation goals. For the rest of this chapter, we will assume that taxonomic uncertainties and management units have been resolved ([Chapter 16](#)), and address genetic issues within management units.



**Fig. 17.1** Flowchart of questions asked in the genetic management of threatened species in the wild.

## Increasing population size

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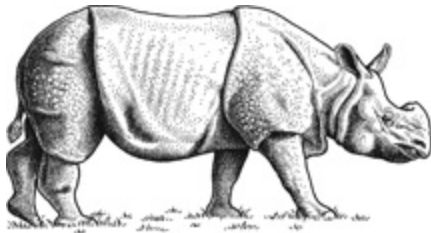
A primary objective in managing wild populations is to increase the sizes of small populations

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The first objective of threatened species management is to increase population size to a point where the population is relatively immune to stochastic (demographic, environmental, catastrophic and genetic) threats. If populations have only recently declined from far larger sizes to  $N_e \sim 50$ , and are rapidly expanded, then the genetic impacts are minimal ([Chapter 8](#)).

The first step in this process is identifying and removing the causes of the decline. This is the domain of wildlife biologists and ecologists. Actions taken include legislative controls on hunting and harvesting, designation of reserves, reduction of pollutants, improvement of habitat quality and eradication of unnatural predators and competitors. These actions typically benefit many native species in the managed region. State reserves now cover about 12% of the land area of the planet, but only 0.5–1% of marine habitats in ~5000 marine protected areas, and both continue to increase rapidly ([Chape \*et al.\* 2003](#); [Young & Randerson 2005](#)). In addition, there are many private reserves and ones maintained by collectives, such as the Nature Conservancy in the USA. Legislative changes in South Africa to vest ownership of wildlife in landowners have led to a substantial expansion of private reserves ([Hayward & Somers 2009](#)). Such procedures have been successful in a diversity of species. For example, Indian rhinoceroses have increased from 27 to about 372 individuals in Chitwan National Park, Nepal, following bans on hunting and designation of the royal hunting reserve as a national park. Similarly, the northern elephant seal has increased from 20–30 to over 175 000 following cessation of hunting and legislative protection ([Weber \*et al.\* 2000](#)). Mauritius kestrel, bald eagle and peregrine falcon

populations have all recovered following control of DDT usage, typically in combination with assisted breeding (Groombridge *et al.* 2000). The Chatham Island black robin has increased from five birds to over 140 following protection, cross-fostering and translocations (Butler & Merton 1992), while the Lord Howe Island woodhen has recovered from 20–30 birds to around 200 following eradication of their primary threat, pigs, and a short-term captive breeding and release program (Brook *et al.* 1997a). The Seychelles warbler has risen from a low of 26–29 birds on Cousin Island between 1959 and 1968 to 1186 birds in 1996 following management and translocation to nearby islands. In plants, recovery has followed legislative protection to minimize harvest, creation of reserves and removal of herbivores (Bowles & Whelan 1994; Primack 2006). Where numbers are extremely small, forms of *ex situ* conservation have been used, followed by reintroductions (Chapters 19 and 20).



Indian rhinoceros

While genetic information may help to alert conservation biologists to the extent of endangerment, management actions to increase population size involve little, or no, genetics. However, recovery in numbers of highly inbred populations can be substantially enhanced following outcrossing (Fig 13.8).

The nene (Hawaiian goose) has been

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Some wild populations are regularly augmented from captive populations, but this is likely to be genetically deleterious in the long term

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subject to a long program of augmentation from captivity, as its wild population is not self-sustaining (Black 1995). Such programs may be counter-productive in the long run, as adaptation to reproduce in captivity is typically deleterious in the wild (Frankham 2008). This is a serious problem in fish where populations that have been in captivity for multiple generations are used to stock wild habitats, but have lower reproductive fitness than residents in the wild (Chapter 20).

## Diagnosing genetic problems

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The main contribution of genetics to management of wild populations has been to diagnose their genetic status

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A necessary precursor to genetic management of wild populations of threatened species is to diagnose their status, by answering the following questions:

- How large is the population ( $N_e$ )?
- Has it experienced significant bottlenecks in the past?
- Has it lost genetic diversity?
- Is it suffering from inbreeding depression?
- What is its geographic distribution?
- Is it genetically fragmented?

Many threatened species including cheetahs, northern hairy-nosed wombats, red-cockaded woodpeckers and Wollemi pines have been



examined to evaluate their status. Where there has been no direct measurement of genetic diversity or inbreeding, we can use theory to predict loss of genetic diversity, as shown in Example 17.1.

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### **Example 17.1 Predicting loss of genetic diversity and inbreeding due to small population sizes**

The population of Florida panthers declined in the early 1900s to population sizes estimated at 6–40 animals ([Box 17.1](#)). Assuming  $N_e/N$  of 0.10, a generation length of 7 years, and conservatively assuming  $N = 40$  from 1920 to 1990, would we have predicted that the Florida panthers had low genetic diversity and were highly inbred in 1990?

From [Equation 11.1](#), and substituting  $N_e = 40 \times 0.1 = 4$  and 70 years = 10 generations, the proportion of original heterozygosity remaining in the population is:

$$\frac{H_t}{H_0} = \left(1 - \frac{1}{2N_e}\right)^t = \left(1 - \frac{1}{2 \times 4}\right)^{10} = 0.875^{10} = 0.26$$

Based on theory alone, we would have predicted that about 74% of its heterozygosity had been lost, a value roughly concordant with the observed loss inferred by comparing Florida panthers with pumas in the Western US ([Box 17.1](#)). From [Equation 12.9](#), the inbreeding coefficient is predicted to be 0.74, a level of inbreeding where deleterious effects would clearly be expected.

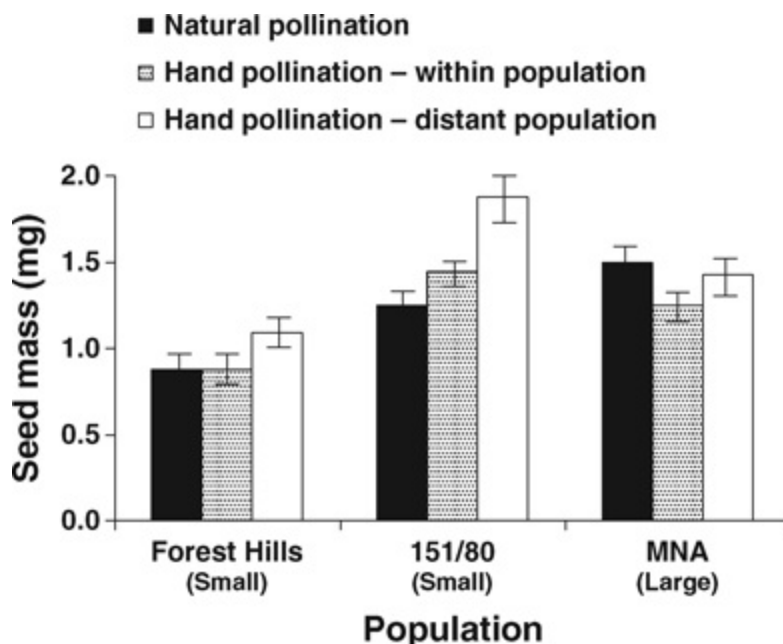
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While there have been many such diagnoses, use of this information to plan conservation management is still in its infancy. Below, we consider the management actions that should be taken to alleviate genetic problems.

## Genetic rescue of small inbred populations by outcrossing

Small, inbred populations can be genetically rescued by introducing unrelated individuals

Introduction of individuals from other populations to improve reproductive fitness and restore genetic diversity (**genetic rescue**) is an effective management strategy in the recovery of small inbred populations with low genetic diversity (Tallmon *et al.* 2004b; Chapter 13). For example, it has led to increases in fitness in natural populations of deer mice, gray wolves, lions, Mexican wolves, greater prairie chickens, Swedish adders, desert topminnow fish and several plant species, including the scarlet gilia (Fig. 17.2).



**Fig. 17.2** Alleviating inbreeding depression in the scarlet gilia plant by outcrossing (after Heschel & Paige 1995). Seed mass per plant for populations at three localities is shown for natural pollination, hand

pollination within populations, and hand pollinations using pollen from distant populations. Small populations (Forest Hills and 151/80) exhibit inbreeding depression for seed mass. The different mean seed masses in the different locations are due to diverse environmental conditions. *Outcrossing improved the seed mass in the small inbred populations, while the large non-inbred MNA population was unaffected by outcrossing.*



Swedish adder

Despite the clear benefits of this procedure, there are very few cases where it is being practiced (see below). Such action is often impeded by exaggerated concerns about outbreeding depression.

## Source of unrelated individuals for genetic augmentation

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Individuals from genetically distantly related populations, or from related inter-fertile taxa, can be used to augment small inbred populations

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The individuals chosen for introduction into inbred populations for recovery of fitness and genetic diversity may be either:

- outbred (if available), or

- inbred but genetically differentiated from the population to which they are being introduced (Spielman & Frankham [1992](#)).

For example, the black-footed rock wallaby has several inbred island populations that could be combined to increase genetic diversity and improve reproductive fitness through hybrid vigour (Eldridge *et al.* [1999](#); [Table 14.1](#)). Where it is unclear from existing information, genetic analyses using multiple nuclear loci (e.g. microsatellites) can be used to resolve the genetic constitutions of populations, as a guide to choosing distinct populations for augmentation.

Where no unrelated individuals of the same taxon are available, individuals from another sub-species can be used to alleviate inbreeding depression and loss of genetic diversity ([Box 17.1](#)). A blight from China severely depleted the American chestnut, and genetic resistance is unknown in the species. The Chinese chestnut species is resistant to the blight and has been crossed with the American species to introduce resistance alleles (Hebard [2006](#)). Further, attempts are being made to engineer disease resistance in the American chestnut by introducing a transgene (Powell *et al.* [2006](#)).

The option of crossing a threatened species to a related species requires extreme caution and must be evaluated on an experimental basis prior to full implementation, to ensure that crossing has beneficial effects in the  $F_1$  and subsequent generations. As genetic differentiation among species varies considerably, crosses among species in some taxa will only be equivalent to crosses between sub-species in other taxa ([Table 16.1](#)). Inter-species crosses have been found to have equal probabilities of having beneficial or deleterious effects on reproductive fitness (Arnold [1997](#)).

## **Management of species with a single population and depleted genetic diversity**

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For species consisting of a single population with reduced genetic diversity, the only options are to improve their environment and minimize risks associated with changed environments (especially disease) and small population size

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From a genetic perspective, the worst situation is where an endangered species exists as a single, inbred, population, with no sub-species or related species available for augmentations. Information on loss of genetic diversity is useful only as an indication of the fragility of the species. For such fragile species, management regimes should be instituted to:

- increase their population size (see above)
- establish populations in several locations (to minimize the risk of catastrophes)
- maximize their reproductive rate by improving their environment (e.g. supplementing food supplies, removing predators and herbivores)
- consider instituting captive breeding or other *ex situ* conservation procedures
- insulate them from environmental changes, including quarantining from introduced diseases, pests, predators and competitors
- monitoring, so that remedial action can be initiated as soon as new environmental threats arise.

For example, the recently discovered and endangered Wollemi pine in Australia has no genetic diversity within and among populations (Peakall *et al.* 2003). The recovery plan calls for (a) restricting access to the populations by keeping their location secret, (b) limiting access to approved people, (c) instituting strict hygiene protocols to avoid introducing disease, (d) fire management and (e) maintaining *ex situ* samples of each plant in botanic gardens (NSW NPWS 1998). Further, commercial propagation has increased population size and reduced stochastic risks, as the species is being spread to many new localities (Jamieson 2005).

The recovery plan for the black-footed ferret calls for creation of a captive population and re-establishment of ten wild populations in different locations, to minimize the risks of disease and other environmental catastrophes ([Chapter 22](#)).

## Genetic management of fragmented populations

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The adverse genetic consequences of population fragmentation can be alleviated by re-establishing historical levels of gene flow among fragments, by improving habitat quality and by re-establishing populations in areas where they have become extinct

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Many threatened species have fragmented habitats, as illustrated for the giant panda in [Fig. 17.3](#). The management options for fragmented populations to maximize genetic diversity and minimize inbreeding and extinction risk are to:

- increase habitat area and quality
- artificially increase the rate of gene flow (e.g. by translocation) to match historical levels
- create habitat corridors, and
- re-establish populations in suitable habitat where they have become extinct.



**Fig. 17.3** Habitat fragmentation for the giant panda in China (after Lu *et al.* 2001).



Giant panda

Gene flow must be re-established to reduce the risk of extinction in

genetically isolated fragments

To alleviate genetic erosion in isolated fragments, gene flow needs to be re-established by moving individuals (**translocation**), or gametes, or by establishing migration **corridors**. The benefits of immigration have been established in many cases ([Chapters 13](#) and [14](#)). In the small scabious plant, between-population crosses had fitnesses 2.5 times that of the within-population crosses (van Treuren *et al.* [1993](#)). Computer projections indicate that immigration will reduce the extinction risks for two small black rhinoceros populations in East Africa ([Box 17.2](#)), and that migration among population fragments will be beneficial in the fragmented tule elk populations in California (McCullough *et al.* [1996](#)).

**Box 17.2 Modelling the effects of inbreeding depression and immigration on the survival of black rhinoceros populations in Kenya (after Dobson *et al.* [1992](#))**

Black rhinoceros populations are threatened across their entire range due, predominantly, to poaching. In East Africa. No single population numbered more than 60 animals when this work was carried out in the early 1990s and the situation is probably worse now. The population is fragmented with no migration among most or all of the fragments.



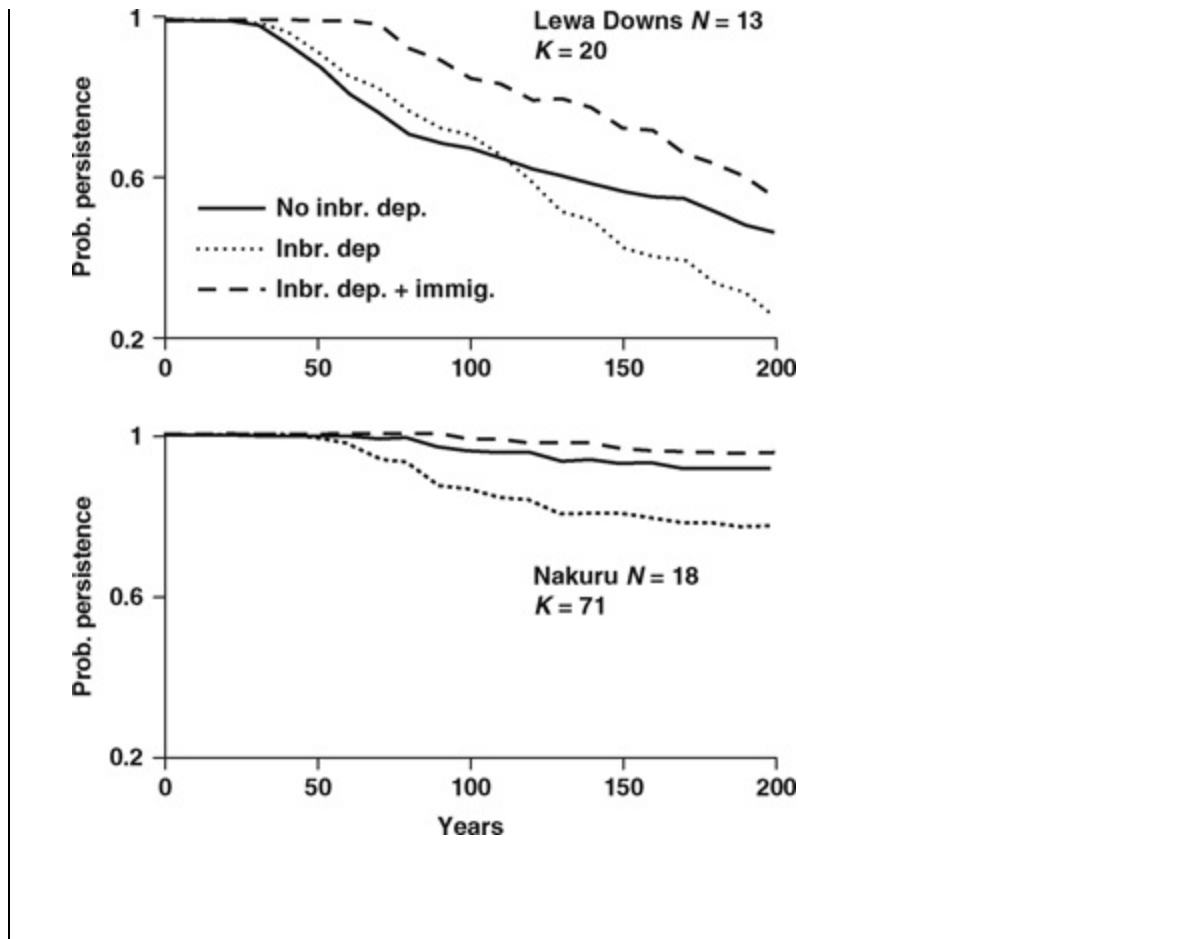
Black rhinoceros



Stochastic computer simulations were used to project the history of each individual from birth to death, including their reproductive output. Input files, using data from sanctuary populations, contained the parentage of each individual, age- and sex-specific rates of survival and reproduction together with rates of immigration of different age and sex classes.

Populations at Lewa Downs and Nakuru National Park, Kenya were modelled for 200 years with (a) no immigration and no inbreeding depression (only demographic and environmental stochasticity), (b) with the addition of inbreeding depression, but with no immigration and (c) with inbreeding depression and immigration of one immigrant every 10 years for the next 50 years. Inbreeding depression on survival was applied at a modest level of 4.0 lethal equivalents per diploid genome. The initial sizes of the two populations were set at their actual sizes, 10 females and three males at Lewa Downs and seven females and 11 males at Nakuru National Park. Carrying capacities were estimated at 20 for Lewa Downs and 71 for Nakuru.

In case (a), stochastic factors led to an extinction risk of >50% after 200 years in the smaller Lewa Downs population, but <10% in the larger Nakuru population. With inbreeding depression (case b), the extinction probabilities rose to 76% and 22%, respectively. With inbreeding depression and immigration, extinction risks were lower, being about 40% and 5% respectively (case c). Consequently, immigration is predicted to reduce extinction risk in these fragmented rhinoceros populations. Additional migration after year 50 would result in further reductions in extinction risks.



Translocation of individuals among populations may be costly, especially for large animals, and carries the risks of injury, disease transmission and behavioural disruption when individuals are released. For example, introduced male lions regularly kill cubs. Further, sexually mature males of many species may kill intruders. The cost of translocations can be reduced by artificial insemination for species where this technique has been perfected. The same care to avoid outbreeding depression, discussed previously, must be exercised in planning translocations.

Corridors among habitat fragments (frequently recommended for non-genetic reasons) can re-establish gene flow among isolated populations. For example, 21 ribbons of habitat have been established between isolated habitat fragments for the golden lion tamarin in Brazil (A. M. de Godoy Teixeira,

pers. comm.). Species vary in their requirements for a corridor to be an effective migration path (Lindenmayer & Nix [1993](#)), but they have been shown to be effective in re-establishing gene flow in several cases (Haddad *et al.* [2003](#)). The most ambitious proposals of this kind are The Wildlands Project in North America, and a related one in Australia, to provide corridors from north to south through the continents (Davis [1992](#); Pulsford *et al.* [2003](#)). The corridors will link existing reserves and surround both reserves and corridors with hospitable buffer zones. The time frame for achieving these is hundreds of years, given the political, social and financial challenges. Nonetheless, such systems are essential if we are to conserve biodiversity in the long term, especially in the face of global climate change.

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Management of fragmented populations is the greatest unanswered challenge in conservation genetics

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Despite the widespread fragmentation of populations of threatened species, we are aware of few cases where augmented gene flow is being used as a practical measure to alleviate inbreeding and loss of genetic diversity in the wild. Notably, it has been used for African wild dogs, lions, elephants and black rhinoceroses in South Africa (Frankham [2009b](#)). Individuals from other populations are being introduced to small populations of endangered red-cockaded woodpeckers, as computer simulations have predicted their likely extinction without augmentation (Haig *et al.* [1993](#)). Other cases include the endangered and inbred Florida panther ([Box 17.1](#)) and the golden lion tamarin ([Box 20.1](#)).

The two known botanical examples involve self-incompatible plants; outcrossing has increased fitness in the threatened lakeside daisy population in Illinois, and in the endangered Mauna Kea silversword (Demauro [1994](#);

Robichaux *et al.* [1997](#)).

## Managing gene flow

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Managing translocations requires continuous genetic monitoring, computer projections to optimize management options, and adaptive management

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Managing gene flow involves considerable complexity as many issues must be addressed, including:

- Which individuals to translocate?
- How many?
- How often?
- From where to where?
- When should translocation begin?
- When should it be ceased?

Answers to these questions require that the population be genetically monitored. Since there are so many variables to optimize, computer projections of the type given in [Box 17.2](#) will often be required to define (and refine) a regime that maintains genetically viable populations with acceptable costs and fits within other management constraints.

## Genetic issues in translocations

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Many species may require translocation to cope with global climate

change, as they may not be able to move unassisted to suitable habitat

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As a consequence of global climate change, many species are projected to have to move to remain in their favoured climatic zones (Thomas *et al.* 2004). Where species have fragmented distributions separated by inhospitable habitat, individuals may require translocation between fragments to ensure population survival. This is particularly the case for species that live on mountain tops or islands, and that have limited dispersal capabilities.

Currently, translocations to establish new populations have low success rates. Only 26% of projects to restore desert fish succeeded, whilst the figures for reptiles and amphibians (for relocations, repatriations and translocations) were 19% and those for mammals and birds 44% (Griffith *et al.* 1989; Dodd & Siegel 1991; Minckley 1995). Consequently, it is important to optimize conditions.

The genetic issues in translocation are

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Individuals used for translocation should be adapted to the release environment and have low inbreeding and high genetic diversity

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adaptation to the release environment, inbreeding and genetic diversity of the released population(s). If no population of the species is adapted to the release environment, crosses should be completed between available populations and  $F_1$  individuals released, as these should have the highest

fitness in the new environment and maximum genetic diversity to allow adaptation to the environment.

Care must be taken with translocations to ensure that the translocated population is representative of the source population. In the threatened Corrigan grevillea, a translocation program sampled 10 of 47 known plants and established 266 translocated plants (Krauss *et al.* 2002). However, only eight clones were represented, 54% of all plants came from a single clone and the translocated population was more inbred and had less genetic diversity than the source plants.

Populations used for translocations should wherever possible have high genetic diversity and low levels of inbreeding. A case of poor choice of populations is provided by the koala in southeastern Australia (Box 17.3). Island populations with low genetic diversity were used for reintroduction, as ample individuals were available from those sources. Genetic issues were ignored and deleterious genetic consequences have resulted. In general, care should be taken when island populations are being considered as source populations for translocation as they typically have low genetic diversity and are inbred (Frankham 1997, 1998; Eldridge *et al.* 1999).

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**Box 17.3 Translocation of koalas in southeastern Australia: a poorly designed program with adverse genetic impacts (Houliden *et al.* 1996; Sherwin *et al.* 2000; Seymour *et al.* 2001)**

The koala is a unique marsupial endemic to eastern Australia. It is both a cultural icon and an important contributor to tourist income. Koalas once ranged from Queensland to Victoria and South Australia (see map), but numbers were reduced by hunting, habitat loss and disease. By the 1930s, koalas inhabited less than 50% of their former range, had disappeared from South Australia, and were nearly extinct in Victoria. The fur trade ceased in the 1930s, when koalas were given legal protection in all States.

They were still common in Queensland, where they subsequently recovered without major assistance.



Koala



Extensive translocations of animals occurred in the southeast. A population was founded from as few as two or three individuals on French Island (FI) in Victoria late in the nineteenth century. It was used since 1923 to directly or indirectly supply 10 000 animals for

translocations to 70 locations in Victoria and South Australia. Animals from French Island were also used to found a population on Kangaroo Island (KI) (18 adult founders plus young) in 1923–25. Kangaroo Island koalas were, in turn, used for further translocations. The current restocked South Australia mainland population has undergone three bottlenecks, mainland Victoria → French Island → Kangaroo Island → mainland South Australia.

The populations in Victoria and South Australia possess about half the genetic diversity found in less-perturbed populations further north. The Kangaroo Island population has the lowest genetic diversity of all surveyed populations. All southeastern populations show similar microsatellite allele frequencies, and similar mtDNA haplotypes, while the more northerly populations exhibit considerable genetic differentiation among populations. The bottlenecked southern populations also have an elevated frequency of males with missing testicles (testicular aplasia), this being worst in the most bottlenecked population on the South Australian mainland.

Loss of genetic diversity and inbreeding depression would have been averted if the French Island population had been founded with more individuals, or if its genetic diversity had been augmented to give it a broader genetic base.

The most efficient strategy to reverse the current problems would be to introduce more genetic diversity into the southeastern populations (both island and mainland) from the nearest populations with high genetic diversity.

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Where there is evidence of adaptive genetic differentiation among extant populations (e.g. many plants and fish), the translocated individuals should come from populations most likely to be adapted to the reintroduction habitat.

## **Re-establishing extinct populations**



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To maintain populations at the largest possible size, extinct populations need to be re-established by translocation from other populations

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If the habitat can still support the species, populations that have become extinct should be re-established from extant populations. Re-establishment should utilize the most genetically diverse population with the highest reproductive fitness in the release environment, or a cross among populations, provided all alternative populations have similar adaptation to the release site.

## **Genetic issues in reserve design**

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Reserves need to be sufficiently large to support the target species, and contain habitat to which the species is adapted. Natural or artificial gene flow must occur among reserves

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Many ecological, genetic and political issues must be balanced in designing nature reserves. Soulé & Simberloff ([1986](#)) suggested that three steps should be involved: (a) identify target or keystone species whose loss would significantly decrease the biodiversity in the reserve, (b) determine the minimum population size needed to guarantee a high probability of long-term survival for these species and (c) estimate the area required to sustain

minimum numbers for these species using known population densities. Further genetic issues in reserve design are:

- is the reserve large enough to support a genetically viable population?
- is the species adapted to the habitat in the reserve?
- should there be one large reserve, or several smaller reserves?

A reserve needs to maintain an  $N_e$  of at least several hundred and an actual size of several thousand, based on the arguments given in [Chapters 15](#) and [22](#).

Should a threatened species be maintained in a single large reserve, or in several smaller reserves? In general, a single large reserve is more desirable from the genetic point of view, if there is a risk that populations in small reserves will become extinct ([Chapter 14](#)). However, protection against catastrophes dictates that more than one reserve is obligatory. The best compromise is to have more than one sizeable reserve, but to ensure that there is natural, or artificial, gene flow among them. In practice, the choice of reserves has often been a haphazard process, determined more by local politics, alternative land uses and the need for reserves to serve multiple purposes (including recreation), than by biological principles.

## Impacts of harvesting

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Harvesting may alter sex-ratio,  $N_e$ , breeding system, generation length, reproductive fitness and gene flow, and typically exacerbates inbreeding and loss of genetic diversity

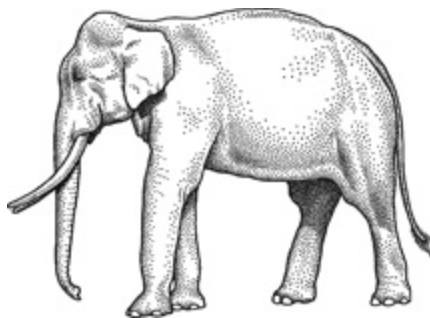
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Many species of wild animals and plants are harvested or poached, e.g. fish, trees, deer and elephants. This may alter effective population size and genetic diversity with deleterious consequences. For example, poaching has impacted on sex-ratio, reproductive rate and effective population size in Asian elephants ([Box 17.4](#)). Further, hunting of moose and white-tailed deer has been projected to severely reduce genetic diversity (Ryman *et al.* [1981](#)). Logging of forests increased the level of inbreeding in a rare tree in Sri Lanka from 15% selfing in primary forest to 35% in logged forest (Murawski *et al.* [1994](#)).

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**Box 17.4 Impact of poaching on sex-ratio, effective population size, reproductive fitness and population viability in Asian elephants (Sukumar *et al.* [1998](#))**

Over the last 20 years poaching for ivory in southern India has decimated the male population, as only male Asian elephants have tusks. In Periyar Reserve there are only six adult males to 605 adult females. From [Equation 11.6](#),  $N_e = 24$ . Further, with such a small number of males, female reproduction rates have declined, as females that do not breed early remain reproductively barren throughout their lives. Harvesting not only affects the sex-ratio and effective size, but also reduces reproductive fitness.



Asian elephant

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Selective harvesting by humans may change the genetic composition and phenotypes of populations

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Selective harvesting may deplete particular phenotypes within populations, e.g. large fish, tusked elephants and deer with large antlers. This selection may change the phenotype of the species, conflicting with forces of natural selection and reducing the overall fitness of the population (Coltman [2008](#)). For example, ivory poaching has increased the frequency of tuskless male elephants in several populations in Africa and Asia (Dobson *et al.* [1992](#); Sukumar *et al.* [1998](#)). Tuskless males may be less successful in finding mates and less able to ward off predators. Hunting primarily targets large-horned bighorn rams and has resulted in a genetic reduction in both horn and body sizes in a Canadian population (Coltman *et al.* [2003](#)).

Commercially exploited fish often show trends towards earlier maturation and smaller sizes, presumably due to fisheries-induced evolution (Kuparinen & Merilä [2007](#); Hutchings & Fraser [2008](#)). For example, earlier maturation is evident in wild exploited grayling fish in Norway and in northern Atlantic cod. Such evolutionary changes may be quite rapid.

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Impacts of selective harvest may be alleviated by changing harvest regimes, or by preserving a portion of the species without harvest

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The obvious solution to these problems is to reduce the selectivity of the harvest, or the intensity of harvest, but these are often very difficult to achieve in practice (Kuparinen & Merilä 2007; Hutchings & Fraser 2008). Harvest of elephant ivory is itself an illegal activity, not subject to regulation. Despite many international agreements, total fish catches are difficult to regulate, as the collapse of many fisheries attests. As harvested species often occur in large, though frequently declining, numbers, an option is to preserve a proportion of the population from harvest. In this way, fully wild stocks are maintained to repopulate harvested areas and minimize the genetic impacts of harvest. Alternatively, setting minimum sizes for fish can reduce the selectivity of the harvest.

Marine protected areas have the potential to minimize the impacts of fish harvest and a large and increasing number are being established. Genetic issues in their design have been considered by Palumbi (2003), but there is little information on their effectiveness. The exploited White Sea bream fish showed higher allelic diversity in the marine protected areas than in non-protected areas in the Mediterranean (Pérez-Ruzafa *et al.* 2006).

## **Genetic management of species that are not outbreeding diploids**

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Species that are not diploid outbreeders require modified genetic management. They often differ from diploid outbreeders in rates of loss of genetic diversity, in differentiation among populations and in their responses to inbreeding

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Many species of plants and some animals are not diploid outbreeders, and

so require modified management regimes. These include species that are:

- asexual (clonal)
- self-fertilizing
- self-incompatible
- gynodioecious
- haplo-diploid
- haploid
- polyploid.

Below we consider their characteristics and the modifications to genetic management that these entail.

## Asexual species

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In asexual species, many individuals may have identical genotypes, so that great care is required to sample the diversity of clones within the species

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Many species of plants are capable of vegetative (clonal) reproduction, via runners, bulbs, corms, etc. Species with exclusively asexual reproduction typically exist as one, or a few **clones**. Genetic diversity may occur between clones, but individuals of the same clone are essentially genetically identical. For example, the endangered triploid shrub King's lomatia and the Meelup mallee tree from Australia each exist as a single clone (Lynch *et al.* 1998; Rossetto *et al.* 1999), while the endangered sea lavender plant from Spain consists of several triploid clones (Palacios & González-Candelas 1997). Partially clonal reproduction is also found in several other threatened plants, including the lakeside daisy, false poison sumac and the Santa Cruz Island

bush mallow in North America (Demauro [1994](#); Hamrick & Godt [1996](#); Rieseberg & Swensen [1996](#)). Asexual reproduction is uncommon in vertebrates, but is found in a range of lizards that have typically arisen from hybridization between species (Fu *et al.* [1998](#)).

In a fully clonal species, inbreeding is not a concern. However, species that only exist as a single clone will have essentially no variation for adapting to environmental changes. This fragility requires management of the type used for the Wollemi pine (see above).

In species that show a mixture of sexual and asexual reproduction, the number of genetically distinct individuals may be far less than the census number of individuals. For example, 53 individuals of the endangered Australian shrub *Haloragodendron lucasii* consisted of only seven clones with reduced genetic diversity compared to an equivalent outbreeding species (Hogbin *et al.* [2000](#)).

Maintenance of genetic diversity in asexually reproducing species requires that the structure of such populations be recognized for *in situ* conservation, in re-establishing extinct populations and in sampling for *ex situ* conservation. The major conservation genetic need is to identify and maintain as many distinct clones as possible.

Mutational accumulation is a much more serious long-term threat to asexual than to sexual populations, emphasizing the need for large populations (Paland & Lynch [2006](#)).

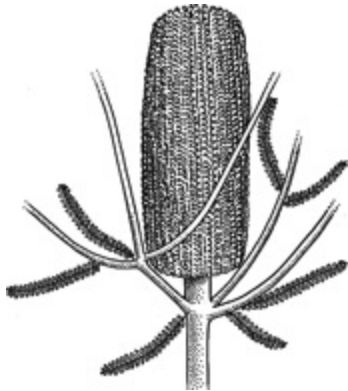
## Self-fertilizing species

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Selfing species typically possess less heterozygosity within populations and more differentiation among populations than outbreeders. Consequently, higher priority should be devoted to preserving diverse

populations than for outbreeders

About 20% of plant species habitually self-fertilize and another 33% do so occasionally. Some invertebrates (e.g. some slugs and snails) also self (Chapter 12). The endangered Malheur wirelettuce is an obligate inbreeder (Falk *et al.* 1996), while Brown's banksia is partially self-fertilizing (Sampson *et al.* 1994).



Brown's banksia

Predominantly self-fertilizing species are typically less heterozygous than outbreeding species, have lower levels of genetic diversity and a higher proportion of their genetic diversity distributed among, rather than within populations (Hamrick & Godt 1989; Charlesworth & Charlesworth 1995; Mable & Adam 2007). For example, *Arabidopsis thaliana* has a high frequency of selfing, is homozygous at most loci and has a level of DNA polymorphism about 50% lower than in another outcrossing species within the same genus (Mitchell-Olds & Schmitt 2006). Compared to outbreeding species, selfing species typically have reduced ability to evolve with environmental change due to both their lower heterozygosity and their lower species-level genetic diversity.

Inbreeding is less of an issue in selfing species, as they typically suffer



lower inbreeding depression than outbreeding species ([Chapter 13](#)). Selfing species show lower loss of genetic diversity in fragmented populations than do outbreeding species (Honnay & Jacquemyn [2007](#)). However, most selfing populations outcross periodically and the opportunity to do so should be maintained in threatened species.

Selfing species therefore require a greater emphasis on conservation of multiple populations than outbreeders.

Mutational accumulation is a much more serious long-term threat to obligate selfing species than to sexual populations, emphasizing the need to maintain large population size (Lynch *et al.* [1995b](#)).

## Self-incompatible species

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Self-incompatible species are particularly susceptible to small population size as they lose self-incompatible alleles, resulting in reduced fitness and increased susceptibility to extinction

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Many species of plants and some snails and slugs have self-incompatibility systems that prevent self-fertilization. Such systems are controlled by one or more loci that, in large populations, have many alleles ([Boxes 9.3](#) and [11.1](#)). These alleles are lost in small populations, reducing mate availability, reducing fitness and increasing the risk of extinction. In addition, these species have the usual susceptibility to inbreeding depression of naturally outbreeding species. If populations become sufficiently small, populations may revert to self-compatibility and to selfing, as has occurred many times in plant evolution (Porcher & Lande [2005](#)). However, the risk of extinction is high during the transition.

Management of self-incompatible species requires even more attention to the deleterious impacts of small population size than required for non-incompatible species. Further, the need to re-establish gene flow among fragmented populations is even more critical.

## Gynodioecious plants

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Gynodioecious species have some plants with female flowers and others with hermaphroditic flowers. They exhibit increased levels of female flowers when inbred, reducing seed set and increasing extinction risk

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Many plant species have mixtures of plants with perfect hermaphrodite flowers (female and male structures) and pistillate flowers (female structures only). After hermaphroditism, this is the commonest reproductive system of flowering plants (Richards 1997). The frequencies of the two flower forms are controlled by the combined effects of cytoplasmic male sterility (determined by mtDNA variants) and dominant alleles at nuclear restorer loci (Schnable & Wise 1998). In small isolated populations, selfing increases the frequency of pistillate plants, often leading to pollen shortage, fewer fertilized ovules and accelerated population decline. This has been observed in *Silene littorea* in Spain (Vilas & García 2006). As they also suffer inbreeding depression, gynodioecious plant species are likely to be more sensitive to population size reduction and inbreeding than hermaphroditic species.

## Haplo-diploid species

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Haplo-diploid species have lower effective population sizes and less genetic diversity than diploid outbreeders and require larger population sizes to retain genetic diversity. They are highly susceptible to extinction from loss of genetic diversity at the sex-determining locus, but are less sensitive to inbreeding depression

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More than 15% of animal species, primarily Hymenoptera (ants, bees and wasps), have haplo-diploid reproduction (Hedrick & Parker 1997). Further, a substantial haploid phase of the life cycle is also found in algae, mosses and ferns where the gametophyte is haploid and the sporophyte is diploid.

In Hymenoptera, females are typically diploid and males haploid. The effective size for a haplo-diploid is only three-quarters that of a diploid of the same census population size, assuming a 1 female : 1 male sex-ratio of reproductives (Table 11.1). Further, only a very small proportion of adults are reproductives in social Hymenoptera, leading to much lower  $N_e/N$  ratios than in diploids. In haplo-diploid species with a single queen mated to a single male per colony, the effective population size is approximately 1.5 times the number of colonies. For example, there are approximately 20 million army ants on Barro Colorado Island, Panama, existing in about 50 colonies each with a single queen and around 400 000 sterile workers. This yields an effective population size of 113 ( $N_e/N \sim 6 \times 10^{-6}$ ) or less, depending on whether queens mate with one or many drones (Chapman & Bourke 2001). Effective population sizes and genetic diversity in haplo-diploid species are therefore generally lower than in diploid insects (Hedrick & Parker 1997).

Haplo-diploid species are expected to exhibit less inbreeding depression than diploids, as selection in haploid males is effective in removing deleterious recessive alleles (Werren 1993; Hedrick & Parker 1997). Haplo-

diploid insects and mites on average suffer less inbreeding depression than diploid insects, as predicted (Henter [2003](#)).

Haplo-diploid species appear to be highly vulnerable to extinction from loss of genetic diversity at the sex-determining locus, an effect reminiscent of effects of loss of self-incompatibility alleles ([Box 17.5](#)).

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**Box 17.5 Death by diploidy: impact of loss of genetic diversity at the sex locus on population viability in haplo-diploid species (Cook & Crozier [1995](#); Pamilo & Crozier [1997](#); Zayed et al. [2004](#); Zayed & Packer [2005](#))**

There are widespread concerns about possible declines in bee pollinator species in many countries (Millennium Ecosystem Assessment [2005b](#); Beiesmeijer *et al.* [2006](#)). Until recently, haplo-diploids were considered less susceptible to the impacts of small population size, as they should suffer less inbreeding depression than diploids, as discussed above. However, evidence now indicates that they may, in fact, be more susceptible than diploids due to the loss of alleles at the sex locus and the production of diploid males.



Orchid bee

Sex determination in most Hymenoptera is controlled by a single complementary sex determining locus (sl-CSD). Heterozygotes are females while haploids are males. However, diploid homozygotes are males that are either sterile or inviable. For example, if a diploid heterozygous female mates with a male sharing one of her CSD alleles,

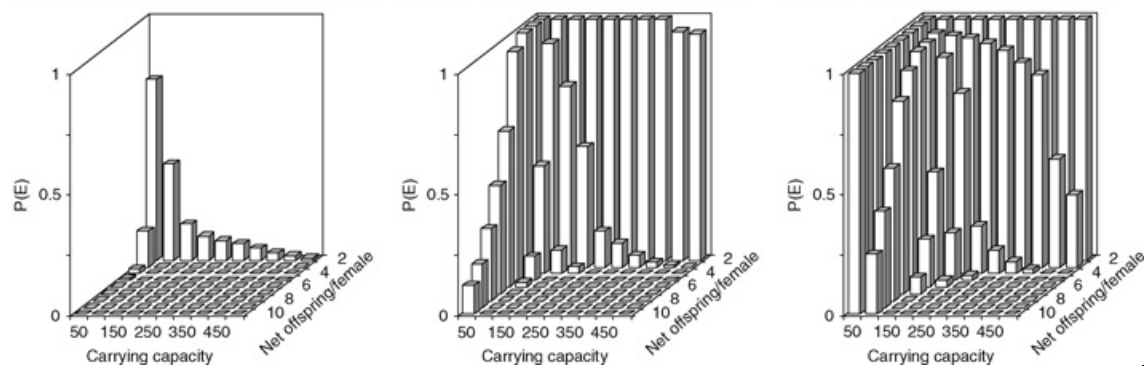
half of her diploid progeny are diploid males, rather than females:

Female ♀		Male ♂
a/b	×	b
	↓	
Diploid progeny		Haploid progeny
½ a/b ♀		½ a ♂
½ <b>b/b diploid ♂</b>		½ b ♂

Large populations maintain many CSD alleles (commonly 9–20) by rare-advantage selection. Diploid males are consequently rare in large populations. In small populations, CSD alleles are lost due to drift, resulting in an elevated frequency of diploid males. For example, *Euglossa imperialis*, the most abundant orchid bee in the lowland forests of Panama, has an average of only 3.1 sex alleles and ~34.3% of males are diploid. Several other Panamanian orchid bees have even higher proportions of diploid males. Loss of CSD alleles and diploid male production is associated with reduced population growth rate in haplo-diploids.

Stochastic computer modelling indicates that loss of CSD alleles in small populations of haplo-diploids greatly elevates extinction risk P(E) (see figures below). Further, it poses a greater threat to population viability than does inbreeding depression in diploid species.

(a) No diploid male production (DMP) (b) DMP with inviable diploid males (c) DMP with viable diploid males



Conservation management of haplo-diploid species will require larger

population sizes than for a diploid species to maintain equivalent proportions of genetic diversity and to prevent extinctions from loss of alleles at the sex locus (Pamilo & Crozier [1997](#); Zayed & Packer [2005](#)).

## Haploids

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Prokaryotic microbes are generally haploid, often reproduce primarily by asexual means, and typically undergo horizontal gene transfer. The major issue is to conserve genotypes adapted to different environments

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Haploidy throughout the life cycle is found in many microbes. Only a few cases of endangered microbial biodiversity have been identified (Souza *et al.* [2006](#)).

We are unaware of any considerations of the conservation genetics of bacteria, but clearly the issues are different from diploid outbreeding eukaryotes. Bacteria are typically haploid and do not suffer inbreeding depression. Microbes reproduce asexually much of the time. Natural selection is typically episodic in microbes with new favourable mutations occurring somewhere in the genome and resulting in selective sweeps that reduce genetic diversity at other loci. Whilst sexual reproduction is relatively rare, bacteria frequently acquire genetic diversity from outside the 'species' (horizontal gene transfer) and replenish lost genetic diversity more readily than do eukaryotes.

Bacteria typically have very large population sizes where genetic drift has limited impact and mutation is effective in replenishing genetic diversity that is lost. Concerns about population size reduction appear to be less than for eukaryotes. In conserving microbes, care is required to ensure that their

taxonomy is resolved and populations adapted to different environments need to be conserved. In general, genetic concerns seem much less for conservation of microbes than for diploid eukaryotes.

## Polyploid species

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Genetic management issues for polyploid species are similar to those for diploid species, but there are fewer concerns about small population size and inbreeding

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Genetic management of outbreeding polyploid species (the majority of angiosperms and ferns) follows the same principles as for diploids with similar breeding systems. However, genetic concerns are generally less serious than for equivalent diploids (Brown & Young 2000; Buza *et al.* 2000). For example, a population of tetraploids contains twice as many copies of each allele as a diploid population of the same effective size, and suffers less loss of genetic diversity and probably less inbreeding depression in small populations than do diploids (Ramsey & Schemske 2002; Chapters 11 and 13). Consequently, polyploids should tolerate lower population sizes than diploids. However, the population sizes required to avoid demographic and environmental stochasticity and catastrophes will be similar to those for diploids (see Chapter 22). Genetic management should avoid hybridization of polyploids and diploids if both occur within the ‘species’.

## Summary

1. Genetic management of wild populations involves increasing population sizes, recovery of small inbred populations, management of fragmented populations and minimizing the genetic impacts of

- harvesting.
2. Small inbred populations with low genetic diversity can be recovered by adding immigrants from other populations (where they exist), and by increasing their sizes to minimize further genetic deterioration.
  3. Fragmented populations with inadequate gene flow require translocations, or creation of corridors, to avoid genetic deterioration. Further, extinct populations should be re-established to maximize population size.
  4. Selective harvesting genetically alters species. Harvest regimes should be designed so that they are as non-selective as possible, or segments of the population should be maintained without harvest.
  5. Species that are not outbreeding diploids have altered susceptibility to loss of genetic diversity and inbreeding depression, and require modified genetic management regimes.

### **Further reading**

Avise & Hamrick (1996) *Conservation Genetics*. Contains case histories on wild management of threatened species.

Coltman (2008) Review on the evolutionary impacts of selective harvest.

Falk & Holsinger (1991) *Genetics and Conservation of Rare Plants*. Book containing papers on wild management of threatened plant species.

Tallmon *et al.* (2004b) Concise review on genetic rescue of small inbred populations.

Trinkel *et al.* (2008) Describes genetic rescue of a small lion population.

Woodford (2000) *The Wollemi Pine*. An interesting popular book on the discovery, conservation and genetics of the Wollemi pine.

World Database of Protected Areas: A comprehensive data set on terrestrial and marine protected areas worldwide. [www.unep-wcmc.org/wdpa/](http://www.unep-wcmc.org/wdpa/)

Zayed & Packer (2005) Documents impacts of loss of sex alleles on extinction risk in Hymenoptera.



## Software

VORTEX: Population viability analysis software that allows comparison of option for management of single or fragmented populations (Lacy *et al.* 2005). [www.vortex9.org/](http://www.vortex9.org/)

## Problems

- 17.1 Recovering inbred populations. How would you alleviate the genetic problems of a moderate sized, but inbred population of euro kangaroos on Barrow Island? Other non-inbred populations exist on the mainland.
- 17.2 Recovering inbred populations. Which population would you choose to recover a small inbred population with a heterozygosity of zero, and a genotype of  $A_1A_1B_1B_1C_1C_1$ ? (a) A large population with genotype  $A_1A_1B_1B_1C_1C_1$ , or (b) a small one with  $A_2A_2B_1B_1C_2C_2$ ?
- 17.3 Recovering inbred populations. Which population would you choose to recover a small inbred population with zero heterozygosity, and a genotype of  $A_1A_1B_1B_1C_1C_1$ ? (a) A large population with zero heterozygosity, or (b) a small one with 2.5% heterozygosity?
- 17.4 Genetic management. How would you address the problem of loss of alleles at the self-incompatibility locus and low seed set in small populations of the endangered grassland daisy (Young *et al.* 2000)? Other populations of this plant exist.
- 17.5 Genetic management. How would you address the problem of fragmentation among isolated island populations of the endangered black-footed rock wallaby?
- 17.6 Managing fragmented populations. Which species in a fragmented habitat is expected to require most translocation to maintain reproductive fitness and genetic diversity in local populations? A bird, a snail or a rodent (assume that all have the same effective sizes in each patch)?
- 17.7 Selective harvesting. What effect would you expect from the

selective harvesting of the largest trees in a forest tree species over many generations?

- 17.8** Genetic management. How would you address the genetic management of the asexual plant King's lomatia in Tasmania, Australia? All individuals belong to a single clone (Lynch *et al.* [1998](#)).
- 17.9** Translocation. What genetic issues do you need to consider in translocation programs?

## Chapter 18 Genetic issues in introduced and invasive species

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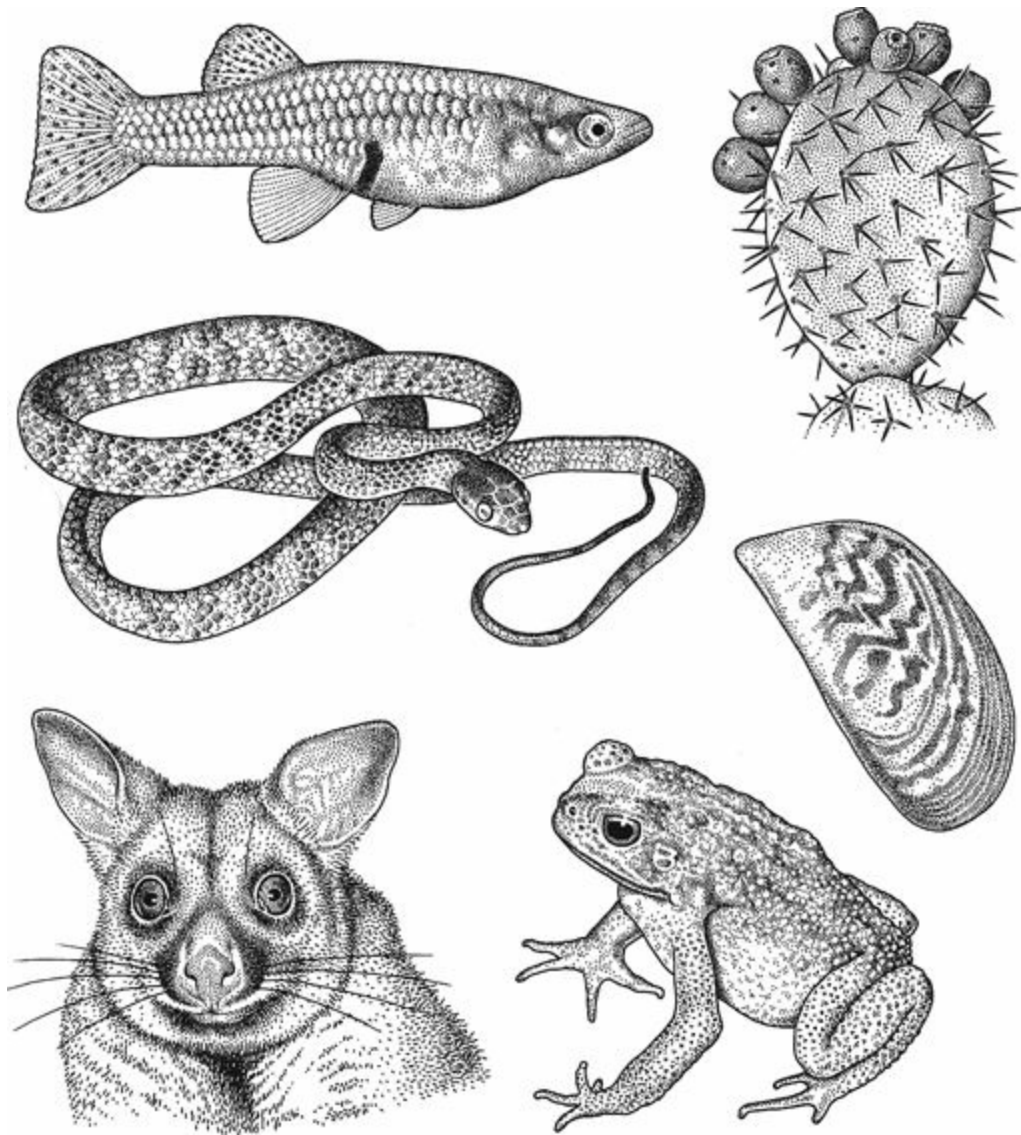
Genetic factors affect the probability of species becoming invasive, their adaptations to local conditions and the prospects of controlling them. Invasive species may also engender evolutionary responses in native species

### **Terms**

Biological control, introgression, invasive species

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A selection of invasive species, clockwise: Gambusia fish, prickly pear cactus, Eurasian zebra mussel, cane toad, brushtail possum, and brown tree snake

## Impact of invasive species on biodiversity

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Introduced species, especially invasive species, are a major threat to biodiversity

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An invasive species introduced into a new range establishes and spreads, typically occupying large areas and affecting many resident species (Allendorf & Lundquist [2003](#)). Inadvertent and deliberate movement of species around the planet is increasing with expanding trade and is likely to accelerate with the extension of ‘free’ trade agreements (Mooney & Cleland [2001](#); McNeely & Schutyser [2003](#)). Global trade has grown from \$US192 billion in 1965 to \$US6 trillion in 2000 with 5 billion tonnes of cargo shipped annually in 165 million containers, mostly by sea (McNeely & Schutyser [2003](#)). The flow of material has overwhelmed customs and quarantine officers, who seem unable to stop the accompanying flood of invasive species. Species are introduced inadvertently as passengers in cargo, via ballast water, deliberately for commercial activity (agriculture, aquaculture, horticulture and the pet trade) and illegally. The introduction of exotic plants into regions of North America is positively related to economic activity in the region (Taylor & Irwin [2004](#)).

The scale of the problem is immense. About 5% of vertebrate species in North America have been introduced from Europe and vice versa, with about 50% of these becoming established, half of which are spreading widely (Jeschke & Strayer [2005](#)). Between 2000 and 2004 over 1 billion animals were legally shipped into the USA; of these, about 80% were fish (Aldhous [2007](#)). Of the 2241 exotic species imported, 302 possess known risk, 246 are known to be invasive and another 61 are associated with disease risks to humans or livestock. There have been more than 4000 plant species introduced into Canada and the USA in the last 400 years and these now represent nearly 20% of the continent's vascular plant biodiversity (Davis

2003). Invasive exotic species are affecting virtually all major rivers, lakes and coastlines in both tropical and temperate zones (McNeely & Schutyser 2003).

Invasive species have many adverse effects on native fauna and flora, including predation, herbivory, competitive displacement and disease. For example, introduced foxes and cats are the major threats to the persistence of small marsupials in Australia. Examples of invasive species include rabbits, cane toads, foxes, domestic cats, prickly pear cactus, bitou bush and rubber vine in Australia, marsupial brushtail possums in New Zealand, Eurasian cheat grass, Eurasian zebra mussels and gypsy moths in the USA, pine trees from Europe and America in South Africa, eucalypt trees from Australia in Africa, Europe and the Americas, brown tree snakes in Guam and other islands, rats, mice, *Gambusia* fish, Argentine ants and fire ants in many countries, and the spread of innumerable diseases and parasites. For example, chytrid fungi have spread around the globe and driven many amphibians to extinction (Mendelson *et al.* 2006). Efforts to come to grips with these problems have led to The Global Invasive Species Program (Mooney 1999).

In many ways, all invasive species are similar to introduced diseases and epidemics. A small number of founders can arrive in new habitat, spread rapidly to establish themselves over large areas and have devastating effects on other species in those areas. The effect on native species is reminiscent of the infection and spread of smallpox by Europeans on native human populations in the New World and elsewhere.

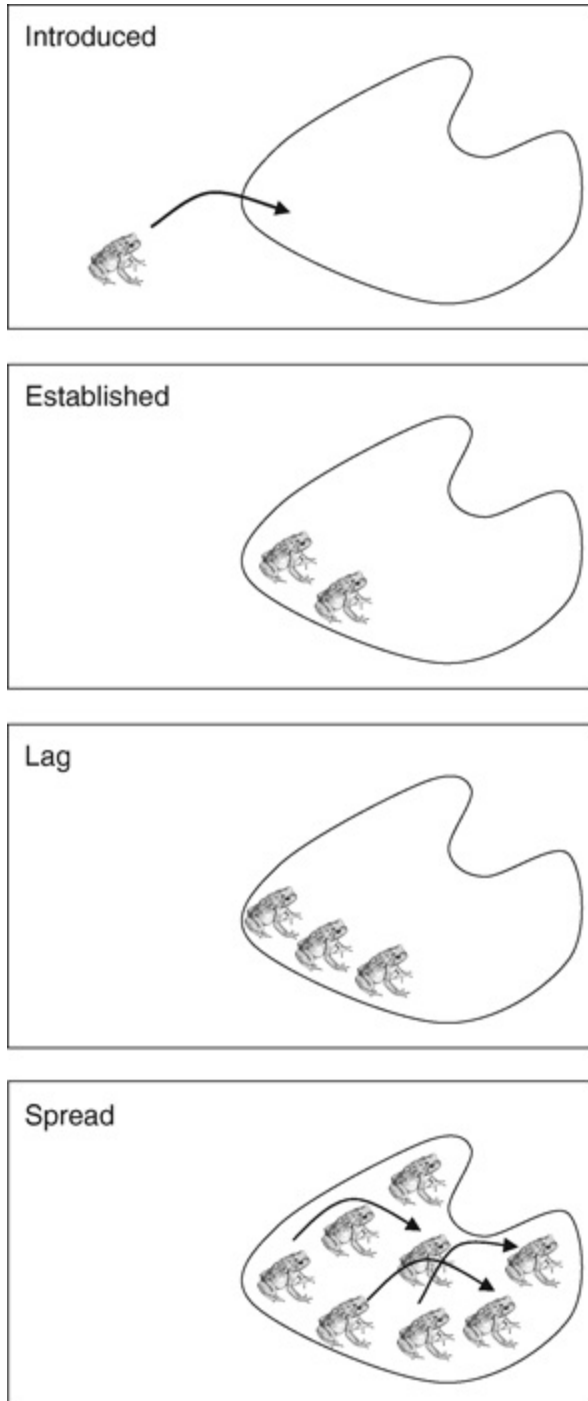
## Phases in establishment of invasive species

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Invasive species typically go through the phases of introduction to a new habitat, establishment, lag and spread

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Figure. 18.1 illustrates the phases in development of an invasive species.



**Fig. 18.1** The path towards an invasive species.

## Introduction and establishment

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The chances of a species spreading in a new habitat depend on its fecundity, dispersal ability and adaptability

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The susceptibility of resident communities to invasion is often, but not always, related to ecosystem disturbance. Propagule pressure is the most important predictor of whether a non-indigenous species will become established (Fyfe [1978](#); Kolar & Lodge [2001](#)). The greater the number of individuals introduced, and the greater the number of release events, the greater the chances of establishment. Thus, species with high fecundity are more likely to become invasive.

Many introductions of exotic species fail. For example, several unsuccessful attempts were made to introduce rabbits into Australia before the successful introduction led to a plague (Fenner & Ratcliffe [1965](#)). Similar examples exist in birds (Fyfe [1978](#)).

## Lag phase

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Demographic factors, genetic adaptation and further introductions to add to genetic diversity all contribute to the lag phase

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The underlying causes of the lag phase are unclear. Demographic factors, genetic adaptation and a requirement for further introductions to augment genetic diversity have been proposed. Efforts to predict the likelihood of an introduced species becoming invasive have usually met with limited success. Strauss *et al.* (2006) found that exotic taxa less related to native species are more likely to be invasive, at least for grass species in California. It is important to determine whether this finding generalizes to other taxa and other geographic regions.

## Population expansion and spread

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The lag phase from introduction to rapid population expansion and invasiveness is highly variable

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If an invasive population experiences a bottleneck at foundation, the impact upon genetic diversity depends on the size of the bottleneck, the subsequent speed of the population size increase and on its breeding system (Barrett *et al.* 2008; Chapter 11). Species that rapidly increase in size following a bottleneck suffer less loss of genetic diversity than those that expand slowly. Patterns of population expansion and spread vary widely.

## Genetic issues in invasion biology

We now discuss how genetic issues affect the chances of introduced species becoming invasive, the prospects of controlling them, evolutionary changes in invasive species and their impact on evolutionary processes in affected native species.

## Source populations for invasive populations

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Comparisons of genetic diversity in potential source populations with that in invading populations can often delineate their origin

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Genetic marker studies can usually identify the source population from which an invasive species has come. For example, the zebra mussel invasion of European and North American waters came from the Black, Caspian and Azov Sea drainages, based upon mtDNA sequence data (May *et al.* [2006](#)). Introduced avian malaria, which has devastated Hawaiian endemic birds, was introduced from the Old World and involved only a single parasite strain of many possible ones (Beadell *et al.* [2006](#)).

## Hybrid invasive species

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Some invasive species arise from hybridization between native and introduced species, with subsequent doubling of chromosome numbers to form fertile allopolyploids

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Ellstrand & Schierenbeck ([2000](#)) list seven allopolyploid invasive species created by hybridization (Hurka *et al.* [2003](#)). For example, common cordgrass (*Spartina anglica*) resulted following introduction of *S. alternifolia* from eastern America into England in shipping ballast, followed by hybridization with native *S. maritima* to produce a sterile hybrid that

subsequently underwent chromosome doubling to form the fertile allotetraploid (Mooney & Cleland [2001](#)). This new species is a vigorous and aggressive perennial that has colonized British and European salt marshes. In the British Isles, ~2% of introduced species are the result of hybridization (Suarez & Tsutsui [2008](#)).



Common cordgrass

## Genetic characteristics of invasive species

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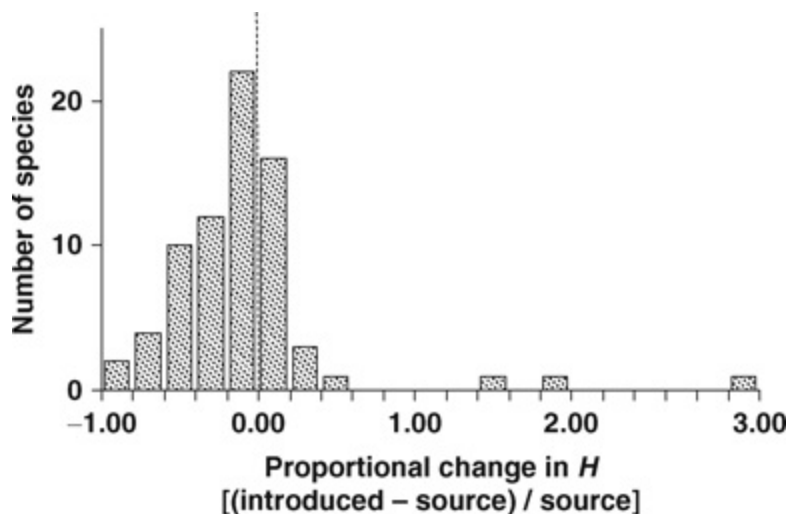
Genetic diversity in invasive species is generally lower than in the source population, but in some species is greater than in any individual population in the native range

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As a small number of propagules are normally involved in introductions, we expect low genetic diversity and reduced ability to evolve. Further, the founder populations will suffer inbreeding depression if they are natural outbreeders. Therefore asexual, naturally inbreeding species and polyploids are predicted to be more likely as invasive species (Allendorf & Lundquist

2003; Hurka *et al.* 2003).

Invasive populations and species usually, but not always, have low genetic variation, based on a meta-analysis (Fig. 18.2). The brown anole only became invasive following repeated introductions into Florida from differentiated Cuban populations. This led to higher heterozygosity in Florida than in any single Cuban population (Kolbe *et al.* 2004). Higher genetic diversity in introduced than in native populations has also been found in other *Anolis* lizards in Florida and Dominican Republic, in chaffinches in New Zealand, hatchery populations of salmonid fish used for reintroductions and for a range of plants including *Raphanus* and *Secale* in California and *Viola* in Germany (Ellstrand & Schierenbeck 2000; Kolbe *et al.* 2007).



**Fig. 18.2** Genetic diversity in invasive populations as a proportion of that in the native source species (Dlugosch & Parker 2008). The dashed line represents no difference. *The majority of invasive populations have reduced genetic diversity.*

## Evolution in introduced species

Invasive species often adapt to their new environment. This may be a major contributor to the evolution of invasiveness

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The lag time between introduction and rapid expansion could involve genetic adaptation to the local environment (Sakai *et al.* 2001). There are many examples of adaptive changes in invasive species (Barrett *et al.* 2008; Suarez & Tsutsui 2008). For example, the Chinese tallow tree evolved increased competitive ability in its introduced range, as did Argentine ants and red fire ants (Allendorf & Lundquist 2003). Evolutionary changes in flowering time may be involved in range expansion of invasive species, as occurred in the purple loosestrife plant in eastern North America (Barrett *et al.* 2008). Cody & Overton (1996) demonstrated evolutionary changes in the dispersal ability of plants that arrived on an island. Initially they had higher dispersal ability, but later evolved lower dispersal ability than the mainland source population. Further, evolutionary changes from outbreeding to selfing or asexuality may occur in invasive species (Barrett *et al.* 2008).

## Evolution of native species in response to introduced species

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The presence of invasive species often causes evolution in native species, either to minimize the adverse impacts of the invaders, or to exploit them as a resource

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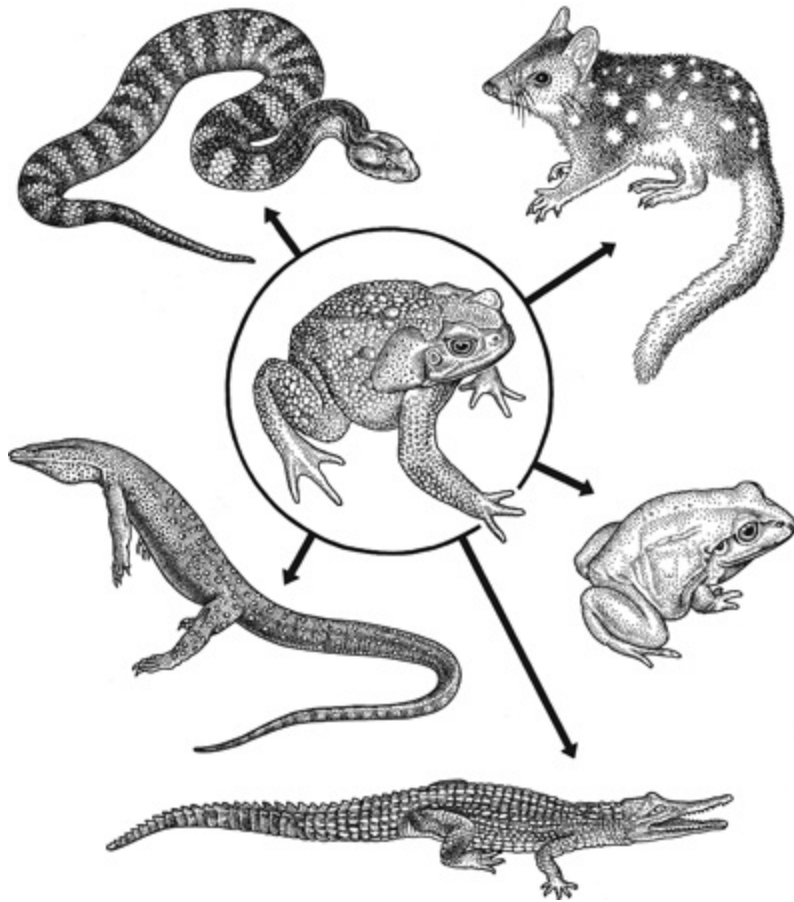
Native species often evolve in response to introduced species (Mooney & Cleland 2001). Native species may be severely impacted by invaders, especially if they are toxic, such as cane toads (Box 18.1). The spread of these toads in Australia has led to a wave of extinctions in predators.

However, some predators have evolved novel characteristics and partially recovered as a consequence. Similarly, blue mussels in southern New England, USA, evolved inducible shell thickening within 15 years of the introduction of the predatory Asian shore crab (Freeman & Byers 2006).

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### **Box 18.1 Impacts of cane toad invasion in Australia (Phillips & Shine 2004, 2006; Phillips et al. 2006)**

The toxic cane toad was introduced into Australia in 1935 to control a pest beetle in sugar cane plantations. It initially spread gradually but is now rapidly moving across the north of the continent. In its path, many native carnivores are being exterminated, including northern quolls (cat-like marsupials), goannas (large varanid lizards), 49 species of snakes, Johnson's crocodile and giant frogs. Some of these species have subsequently shown partial recovery. This probably involves adaptive changes, whether in behaviour, morphology or toxin resistance. Two species of snakes have evolved changes in head size such that they eat fewer or smaller toads, while two species of snake at low risk from toads have shown no such change (Phillips & Shine 2004). Further, black snakes have evolved increased resistance to the toad toxin and a decreased preference for toads as prey (Phillips & Shine 2006). If a toxin resistance gene can be identified, it would be possible to cross genetically resistant individual predators from behind the invasion front with individuals of the same species ahead of the toad front to reduce the impact of toads on population sizes and viability.



Cane toad in centre, with species that it affects, including clockwise, death adder, northern quoll, giant frog, Johnson's crocodile and goanna

Cane toads had a long lag period before they became invasive. However, the rate of advance has increased by about five-fold over 60 years. Invasion front toads move further, more often and in straighter lines than toads from older populations. Leg length relative to body length has increased over time and this is related to increased speed of movement.

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Native species may evolve changed host specificity to exploit an introduced species. Australian seed-feeding soapberry bugs now attack introduced forest-invading balloon vines and have evolved longer mouthparts to better exploit this new host (Carroll [2008](#)). In contrast, the North American

soapberry bug has evolved shorter mouthparts following colonization of the introduced Asian flamegold tree (Lee [2002](#); Carroll [2008](#)).

A complex case of the effects of introgression and hybridization is provided by native tephritid fruit flies that moved to introduced, mainly hybrid and introgressed, honeysuckle plants in northeastern USA (Schwarz *et al.* [2005](#)). The tephritid flies that host shifted to the invasive host plant were found to be a new diploid species, formed from hybridization of two native fly species.

## Control of invasive species

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The most effective control of invasive species is to prevent their establishment, or to eradicate them soon after their arrival. The longer they are established, the harder they are to eliminate

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The ideal control of invasive species is to prevent their introduction into new areas. For example, many marine invasive species have been spread in ship ballast water. Procedures have now been devised to reduce this risk by flushing ballast water with nitrogen to kill any marine organisms contained in the water (McNeely & Schutyser [2003](#)). This procedure also reduces the rate of corrosion in ships, increasing the chance that it may be implemented on a widespread basis.

It is critical to commence control procedures as soon as an invasive species is detected. This is advisable for both demographic and genetic reasons. As adaptation to the new environment is expected, introduced species are more likely to be susceptible to early than to late control. Populations with low



genetic diversity are easier to control biologically (Burdon & Marshall [1981](#)) and probably also by chemicals.

## Biological control

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For many invasive species, particularly plants, biological control is the only feasible method of control

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**Biological control** is the use of introduced parasites, predators and pathogens to reduce invaders' density below what would occur in their absence (McFadyen [1998](#)). For many invasive species, especially plants, it may be the only feasible option for control. McFadyen ([1998](#)) concluded that biological control of weeds using imported insects and pathogens is safe, environmentally sound and cost effective. For example, highly successful biological control of the exotic and invasive prickly pear cactus in Australia was achieved by introduction of the cactoblastis moth. Similarly, the water weed salvinia has been controlled in many areas by introduced weevils. However, introduction of species for biological control carries the risk that the control agent may spread to other non-target species. This has occurred with cactoblastis moths in the USA, where they are now attacking threatened species of cactus (McFadyen [1998](#)). This is the only known case of a serious threat to an endangered species from a weed biocontrol agent (McFadyen [1998](#)). The cane toad introduction in Australia has become a national disaster ([Box 18.1](#)). Similarly, mongooses have been introduced in many places to kill rats, but have frequently become major predators of native species.

Introduction of disease agents may provide temporary control of pest species, but the invasive species is likely to evolve resistance. This occurred when myxomatosis was introduced into Australia to control rabbits ([Box](#)

6.1). Rabbits in Australia illustrate the difficulties of controlling invasive species. Control procedures have included gassing, poisoning with arsenic and sodium fluoroacetate (1080), ploughing warrens, shooting, hunting with ferrets, building exclusion fences many thousands of kilometres long, and introduction of myxomatosis and calici viruses. However, the rabbits still prosper.

## Immunocontraception

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Major efforts are under way to control introduced vertebrate pests by immunocontraception

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Immunocontraception involves immunizing individuals against their own sperm, eggs or reproductive hormones (Grant & Malin 1997). These efforts are targeted on rabbits, foxes and feral cats in Australia, Australian brushtail possums in New Zealand, and island horse populations in the USA (Box 18.2). The proposal is to identify sex-specific molecules on the sex cells, produce large quantities of these antigen proteins and deliver them to the pest species via either viruses or baits. These programs are controversial, as some critics predict that natural selection will rapidly render any control ineffective (Cooper 1999; Cooper & Herbert 2001; Magiafoglou *et al.* 2003).

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### **Box 18.2 Immunocontraceptive control of Assateague Island horses (Zimmerman *et al.* 2006)**

Feral horses have lived on Assateague Island (a 37-mile barrier island off the Atlantic coast of Virginia and Maryland, USA) since the 1600s. The

population was probably established by early settlers and, until recently, has experienced sufficient gene flow from the mainland population to preserve genetic diversity and avoid inbreeding depression. It is currently managed as two herds, one in Virginia which is managed through annual round-ups and auctions, and one in Maryland whose population has received 'hands-off' management, with occasional removal of individuals that caused problems for visitors to the island.

In 1968, the Maryland herd was reduced to only 28 individuals by a hurricane, but grew to 166 horses in 1994. While the horses are neither endangered nor under genetic stress, they now require intensive management, as their density has grown to a level with significant impacts on endangered species on the island and to ecological processes. A contraceptive population control program, using the immunocontraceptive porcine zona pellucida (PZP), was initiated in 1994. However, without the stress of reproduction, contracepted mares lived significantly longer than expected and the herd has not been reduced as rapidly as initially desired.

In 2006 the US National Park Service (NPS) and the Conservation Breeding Specialist Group (CBSG) conducted a population and habitat viability workshop to evaluate alternative management plans. The competing interests addressed were: (1) managing a horse population in balance with the delicate island ecosystem (with its endangered species and rare plant communities); and (2) maintaining a herd large enough to allow visitors to routinely, but safely, view the free-roaming animals. Capture and removal of horses from the island is not acceptable from animal welfare and public relations perspectives. Therefore, PVA computer modelling ([Chapter 22](#)) was used to project short-term reductions in population size using only contraception, and to evaluate the impact of different target sizes on accumulation of inbreeding. Under the existing management practices using immunocontraception, the model predicts that the population will decline to ~100 animals in about 5 to 6 years, 80 horses in 7 to 8 years and 50 animals within 9 to 10 years. Inbreeding will accumulate slowly over the next 50 years to levels that are unlikely to result in significant reductions of population viability. The modelling resulted in a decision to set a short-term target of 80–100 horses, with an understanding that this target will be adjusted through adaptive management as the impacts of changing population size on horse and ecosystem health are monitored.

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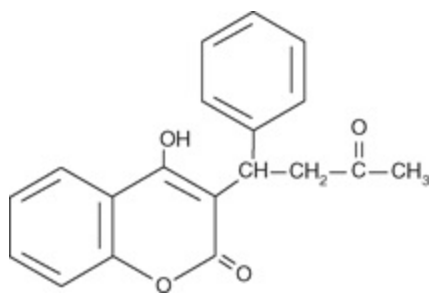
An important tool in evaluating the feasibility of immunocontraception is population viability analysis, as illustrated in [Box 18.2 \(Chapter 22\)](#).

## Evolution of resistance to biocides

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The development of genetic resistance to biocontrol agents is a universal phenomenon

Control of introduced invasive species often involves poisoning with biocides. However, use of biocides frequently fails in the long term due to evolution of resistance. This has occurred for insecticides in hundreds of species of insect, for herbicides in 183 weed species, and for rodenticides such as warfarin, in mice and rats (McKenzie [1996](#); Pelz *et al.* [2005](#); Heap [2007](#)).



Warfarin

Sodium fluoroacetate (1080) is the primary method of control for introduced foxes in Australia and brushtail possums in New Zealand.

Resistance to 1080 is expected to evolve. Native species in Western Australia are already resistant to this compound, as sodium fluoroacetate occurs in several native legumes in Western Australia (Cooper & Hebert [2001](#)). Rabbits in Australia have been poisoned with 1080 for over 50 years and are now exhibiting increased resistance (Twigg *et al.* [2002](#)).

## Genetic control of invasive species

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A range of different genetic control methods have been proposed for invasive pest species and some have been attempted in the field. Only the sterile insect release method has met with substantial success

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Various proposals have been made to use genetics to control invasive pest species (Whitten & Foster [1975](#)). The most successful of these has been sterile male insect release for species where females mate only once. Such programs include those for screwworms, melon and oriental fruit flies. Laboratory-reared males are sterilized using irradiation and large numbers released so that they outnumber field males by about 10 : 1 (Robinson [2002](#)).

Compound autosomal chromosome strains have been proposed as a genetic control method, as they produce no progeny in crosses with normal strains, resulting in an unstable equilibrium ([Box 18.3](#)). The normal strain can be replaced by the compound strain by introducing more than four times the number of compound individuals relative to normal ones. This could be used to replace an insecticide-resistant strain with a susceptible one, or a population of mosquitoes that transmit disease with one that is refractory to disease. Use of an excess of a compound chromosome strain of flies successfully eliminated a normal chromosome strain in laboratory experiments in fruit flies (Foster *et al.* [1972](#)). Such programs were trialled in

the Australian sheep blowfly, but later disbanded (Foster *et al.* 1985, 1991). Heterozygotes for translocations are partially sterile and also result in an unstable equilibrium. Consequently, release of translocation-bearing individuals at a frequency exceeding the unstable equilibrium frequency changes the genetic composition of the population. The use of meiotic drive (transmission distortion) was also considered, but rejected on the grounds that chromosomes with such characteristics had deleterious fitness consequences and would be selected against. Generally, laboratory strains produced to alter the genetic composition of wild populations have a history of low reproductive fitness, making such approaches difficult or impractical.

### **Box 18.3 Replacement of normal chromosome strains with compound chromosome strains by exceeding the unstable equilibrium point (Foster *et al.* 1972)**

The diagram below illustrates the normal fruit fly genome and a compound chromosome strain where the two left arms of chromosome II are attached to one centromere and the two right arms to a different centromere.



During meiosis in the compound strain, both chromosomes can segregate to one pole and none to the other, or one to each pole. Thus the compound strain only has about  $\frac{1}{4}$  of its zygotes viable (+) with 2 left and 2 right arms, whilst the other  $\frac{3}{4}$  have unbalanced chromosome complements (–)

Eggs	Sperm			
	2L.2L	2R.2R	2L.2L -	2R.2R -
2L.2L 2R.2R	-	-	-	+
2L.2L -	-	-	+	-
2R.2R -	-	+	-	-
--	+	-	-	-

Crossing the normal and compound strain yields no viable progeny, as all progeny have unbalanced chromosome complements (-), as illustrated below.

	Compound strain gametes			
	2L.2L	2R.2R	2L.2L -	2R.2R -
Normal gamete 2L.2R	-	-	-	-

When a population is formed with proportions  $p$  of normal strain (NN) and  $q$  of compound strain (CC), random mating results in the following frequencies and fitnesses

	NN	NC	CC	Total
Zygotic frequencies	$p^2$	$2pq$	$q^2$	1.0
Relative fitnesses	1	0	$1/4$	
After selection	$p^2$	0	$1/4q^2$	$p^2 + 1/4q^2$
Adjusting for total	$\frac{p^2}{p^2 + 1/4q^2}$	0	$\frac{1/4q^2}{p^2 + 1/4q^2}$	1.0

New frequency  $p_1 = \frac{p^2}{p^2 + 1/4q^2}$  Change in frequency  $\Delta p = p_1 - p = \frac{p^2 - p(p^2 + 1/4q^2)}{p^2 + 1/4q^2} = \frac{p^2 - p^3 - 1/4pq^2}{p^2 + 1/4q^2} = \frac{p^2(1-p) - 1/4pq^2}{p^2 + 1/4q^2} = \frac{p^2q - 1/4pq^2}{p^2 + 1/4q^2} = \frac{pq(p - 1/4q)}{p^2 + 1/4q^2}$  At equilibrium,  $\Delta p = 0$ , so  $\Delta p = 0$ , so  $\hat{p} = 1/4\hat{q}$  and substituting  $p = 1 - q$  yields  $p = 1 - q$  yields  $\hat{q} = \frac{4}{5}$

This is an unstable equilibrium, as  $\Delta p$  is negative when  $p$  is below the equilibrium (the population goes to fixation of CC) and positive when  $p$  is above the equilibrium (population goes to fixation of NN). Thus, the normal chromosome strain can be replaced by flooding it with more than four times as many compound strain individuals as there are normal ones.

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A control program is under way to introduce a daughterless gene to distort sex-ratios towards males in exotic carp in Australia, a species causing major problems in inland waterways (Thresher & Bax [2003](#)). A transgene that blocks the aromatase locus inhibits the conversion of androgen to oestrogen and converts females into males. This has been achieved in a zebrafish model. The program would involve continued release of large numbers of transgenic male carp over a long period. Hatchery-bred transgenic males will need to be competitive with wild males for the program to be successful.

### **Defining eradication units and monitoring eradication attempts**

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Genetic markers can be used to define eradication units and to monitor eradication programs

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Eradication of invasive brown rats on South Georgia Island (4000 km<sup>2</sup>) would be an order of magnitude larger than any previous successful rat eradication program. However, the rat populations are separated by glaciers into more manageable-sized units and microsatellite analyses showed that populations are differentiated and show little, if any, gene flow (Robertson & Gemmell [2004](#)). Thus, eradication can proceed in stages to eliminate rats in each area of the island, with low risk of recolonization.

Genetic studies have also delineated the causes of failed eradication attempts. Microsatellite analyses and assignment tests revealed that one failure of ship rat eradications on islets off the Martinique coast was due to



incomplete eradication and another to reinvasion (Abdelkrim *et al.* [2007](#)).

## Introgression and hybridization

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Some species are endangered due to genetic ‘swamping’ resulting from interbreeding with common related species. Genetic markers can be used to detect introgression and hybrid individuals

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**Introgression** is the flow of alleles from one species or sub-species to another. It is a threat to the genetic integrity of many species including canids, ducks, fish and plants (Rhymer & Simberloff [1996](#); Ellstrand [2003](#); Randi [2008](#)). Typically, hybridizations occur when humans introduce exotic species into the range of related species (Ellstrand *et al.* [1999](#)), or alter habitat so that previously isolated species are now in secondary contact. For example, rainbow trout have been introduced into the habitats of North American salmonids and hybridize with several native species. Golden and capped langurs were previously isolated by canyons in the Himalayas, but a recently constructed bridge has allowed contact and hybridization (Wangchuk [2005](#)).

Introgression can be detected using a wide range of genetic markers, including allozymes, microsatellites, DNA fingerprints, RAPDs and, where appropriate, chromosomes. Allozyme analyses of captive Asiatic lions indicated that they carried alleles from African lions (O'Brien [1994](#)). Endangered Florida panthers consist of two populations, one of which resulted from introgression with a South American sub-species ([Box 17.1](#)). Microsatellite analyses reveal that females of the highly endangered Ethiopian wolf hybridize with male domestic dogs, with the levels varying in different populations ([Box 4.2](#) and Example 7.3). Conversely, microsatellite

data on Mexican wolf populations showed no evidence of introgression from dogs or coyotes (Hedrick *et al.* 1997; [Chapter 21](#)). A particularly deleterious form of hybridization is that between related diploid and tetraploid populations, resulting in sterile triploids. This has been reported in an endangered grassland daisy in Australia (Young & Murray 2000).

## Introgression from transgenics

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Transgenes may move from genetically engineered domestic plants and animals to related wild species. The impact will depend upon the loci contained in the transgenics and, in some cases, these may have deleterious impacts on wild species

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Gene flow from genetically engineered domestic animals and plants to wild species provides a special case of introgression. Approximately one-eighth of the world's croplands are planted with transgenics (Raven 2005). To date, there is limited evidence on the impacts of these, but potential problems have been discussed by Ellstrand (2003). The crucial issue about transgenes is the sequence that they contain and its effects, not whether the gene is moved by genetic engineering, as natural 'genetic engineering' already occurs in a range of species. Transgenes containing herbicide resistance genes, such as those for glyphosate (Roundup<sup>®</sup>), have been widely used in crop plants (Ellstrand 2003). These transgenes could potentially move to related weed species with major economic implications. However, most transgenics grow in areas lacking related wild species and adverse conservation effects do not appear to be likely. Nonetheless, glyphosate-resistant transgenes have been found in wild field mustard plants that received pollen from nearby, closely related, cultivated transgenic canola in Canada (Warwick *et al.* 2003, 2008). The impacts on wild biodiversity have not been evaluated. Creeping bentgrass containing a glyphosate-resistance transgene has recently escaped

into the wild in the USA, the first such case identified (Reichman *et al.* 2006). This perennial species is widely used on golf courses and has wild and weedy relatives. The full impacts of the escape of the transgene-containing form are not yet clear.

Considerable publicity attended the discovery that maize pollen carrying a transgene for production of Bt toxin were toxic to monarch butterflies. However, a later field study found no adverse effects of the Bt maize on lepidopteran larvae living on accompanying weeds (Gathman *et al.* 2006). A meta-analysis of 42 field experiments indicates that non-target invertebrates are generally more abundant in Bt cotton and Bt maize fields than in non-transgenic fields managed with insecticides (Marvier *et al.* 2007). However, in comparison with insecticide-free control fields, certain non-target taxa are less abundant in Bt fields. The most credible threats to biodiversity from plant transgenics are probably the movement of transgenes for environmental tolerances (e.g. cold or heat tolerance) from crop plants to invasive weeds, allowing the invasives to expand their range and further impact biodiversity.

Fish transgenics containing growth hormone genes are of greater concern, as they result in larger individuals (up to more than seven-fold larger in coho salmon) that outcompete and cannibalize normal fish. Transgenic males are more competitive in matings than native fish of the same species, but progeny carrying the transgene suffer lower survival (Devlin *et al.* 2004; Howard *et al.* 2004). The overall impact of the transgene depended upon food levels. At low food levels populations containing transgenes experienced population crashes and/or complete extinctions, while groups containing only normal fish had good survival and their population biomass continued to increase (Devlin *et al.* 2004).

## Alleviating introgression

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Introgression can be alleviated by removing the hybridizing species, by
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eliminating hybrid individuals, or by expanding the number of ‘pure’ individuals

The Catalina mahogany ([Box 18.4](#)) provides an example of management of a species subject to hybridization. The major thrust of this program involves protection of remaining plants and increasing their numbers. Genetic information was critical in defining the problem, and in identifying hybrid individuals, but had a limited role in the recovery process.

**Box 18.4 Catalina mahogany: identification of hybridization with a related species and institution of a recovery plan (Rieseberg & Swensen [1996](#))**

The Catalina mahogany is a small endangered tree restricted almost entirely to a single gully on Santa Catalina Island in the Channel Islands off California. The species is extremely rare, having declined from 40 to 11 plants by 1996, largely as a consequence of grazing by introduced goats. An inventory in the late 1970s identified several individuals that resembled mountain mahogany (a separate species), or were intermediate. Genetic studies using allozymes and RAPDs identified six of 11 adult trees as ‘true’ Catalina mahogany, and the other five as hybrids with mountain mahogany.



Catalina mahogany

Management began with fencing off two 'true' Catalina mahogany trees, resulting in the production of about 70 seedlings that were mostly allozymically 'pure'. Several cuttings from the 'pure' trees have been propagated and 16 of these have been planted on the island. A major problem remains the grazing and rooting of goats, pigs and recently introduced bison and mule deer, despite efforts to control them.

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Options for addressing introgression include eliminating the introduced species, or translocating 'pure' individuals into isolated regions or into captivity. Success for these options is hard to achieve. It will not be practical to remove all domestic dogs from the habitat of the Ethiopian wolf. For native salmon of the Pacific Northwest, other salmonids have already been introduced. In theory, fish could be exterminated in closed lakes and restocking carried out with 'pure' native fish. However, control of fish dispersal is notoriously difficult, as fishermen often undertake illegal stocking.

## Summary

1. Invasive species have major adverse effects on biodiversity.
2. Genetic diversity is usually lower in invasive species than in their source population. However, it is sometimes greater than in any individual population in the native range, due to repeated introductions from different source populations.
3. Some invasive species arise from hybridization between native and introduced species and doubling of chromosome numbers to form fertile allopolyploids.
4. Adaptation to the introduced environment often occurs in invasive species and may be a major contributor to the evolution of invasiveness.
5. Invasive species often cause native species to evolve in response to their presence, either to minimize the adverse impacts of the invasive

- species, or to exploit the invasive species as a resource.
6. The most effective control of invasive species is to prevent their establishment, or to eradicate them as soon as they arrive. The longer they are established, the harder they are to eliminate.
  7. Hybridization with common related species is a threat to some mammals, birds and plants, and numerous fish species. Management is required to minimize hybridization by reducing the common species or by protecting 'pure' populations.

### **Further reading**

Ellstrand (2003) *Dangerous Liaisons?* Book on introgression in plants and potential genetic impacts of transgenic plants.

IUCN Invasive Species Specialist Group: Website with material on invasive species, including the *Global Invasive Species Database*, and information on the Global Invasive Species Programme. [www.issg.org/](http://www.issg.org/)

Lee (2002) Brief review on the evolutionary genetics of invasive species.

*Molecular Ecology* (January 2008) Special issue on evolutionary change in human-altered environments; has several relevant papers on invasive species.

NEMESIS: National Exotic Marine and Estuarine Species Information System; has information on exotic species in coastal marine waters of the USA. <http://invasions.si.edu/nemesis/>

Phillips & Shine (2004) Documents adaptation in two Australian snakes to the exotic invasive cane toad by changes in head to body size.

Phillips *et al.* (2006) Documents increased rate of invasion due to increased relative leg length in cane toads.

Sakai *et al.* (2001) Review on the population biology of invasive species.

### **Software**

HYBRIDLAB: Program for generating simulated hybrids to determine

statistical power for hybrid detection (Nielsen *et al.* 2006).  
[www.difres.dk/ffi/uk/populationgenetic/hybridlab/index.asp](http://www.difres.dk/ffi/uk/populationgenetic/hybridlab/index.asp)

NEWHYBRID: Program to detect hybrids based on genetic data  
(Anderson & Thompson 2002).  
<http://ib.berkeley.edu/labs/slatkin/eriq/software/>

## Problems

- 18.1 Control of invasive species. How would you suggest attempting to control the invasive cane toad in Australia?
- 18.2 Evolution of biocide resistance in an invasive species. What determines the likelihood of foxes in Australia evolving resistance to 1080 poison?
- 18.3 Impacts of transgenes. Discuss the potential impacts of transgenes on biodiversity
- 18.4 Unstable equilibrium for a compound chromosome strain. If a compound chromosome strain has a relative fitness of only 0.1 that of the normal strain (as has been observed in some cases), what is the unstable equilibrium frequency? What multiple of  $N$ , the number of normal individuals of the compound strain, must be introduced to replace the normal strain in this case?
- 18.5 Unstable equilibrium for a translocation. What is the equilibrium frequency for a population formed by mixing a translocation strain and a normal chromosome strain at frequencies of  $p$  with  $q$ , respectively, if the TT, TN and NN zygotes have relative fitnesses of 1, 0.5 and 1?

## Chapter 19 Genetic management of captive populations

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Captive breeding permits conservation of species that are incapable of surviving in their natural habitats. Endangered species in captivity are managed to retain high levels of genetic diversity over long periods, frequently by minimizing mean kinship

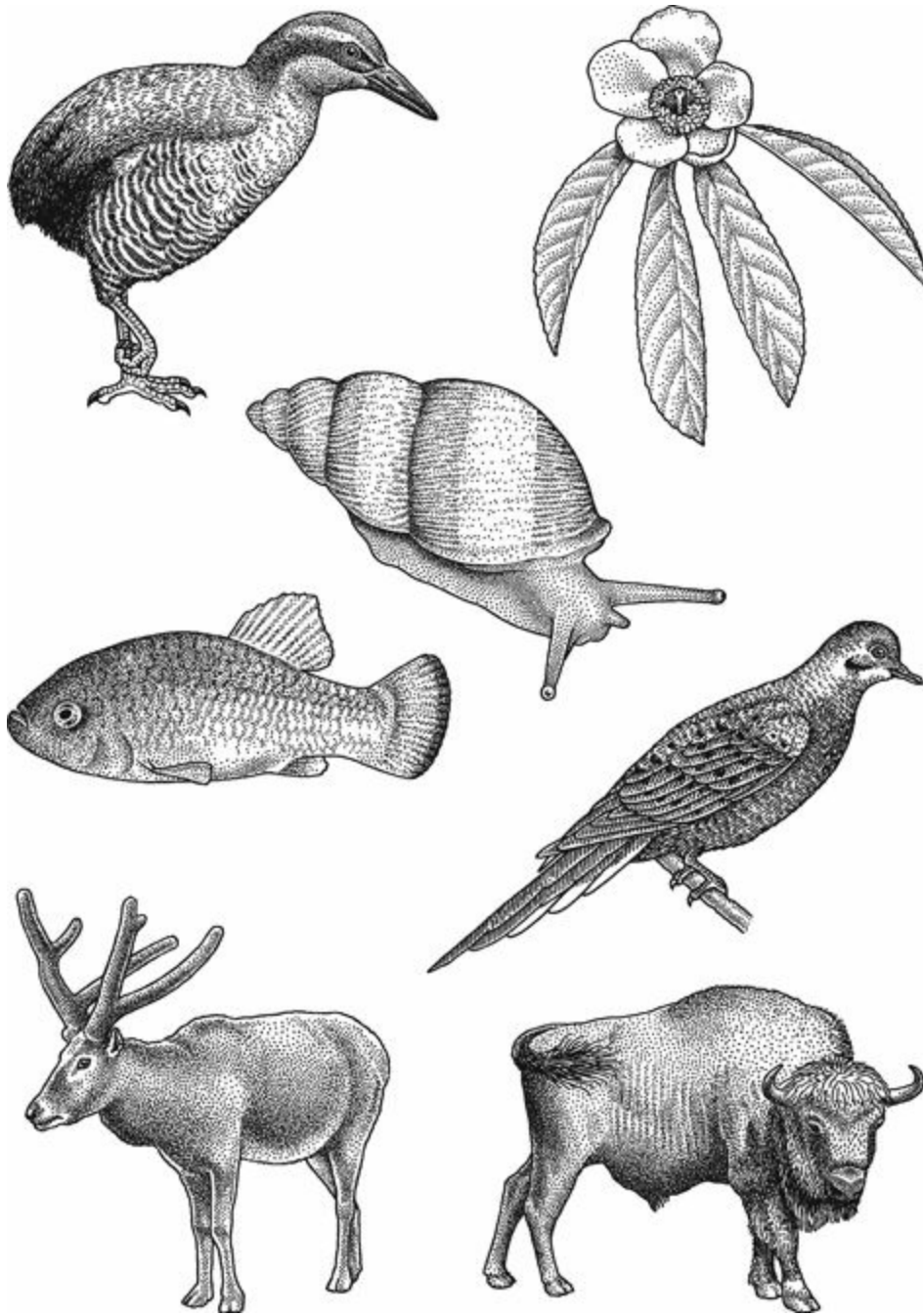
### Terms

Coancestry, *ex situ* conservation, founder effect, gene drop, genome resource bank, *in situ* conservation, kinship, mean kinship, reintroduction, studbook

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A selection of endangered species that have been saved from extinction by captive breeding: Guam rail, Franklin tree (North America), *Partula* snail (Tahiti), Potosi pupfish (Mexico), Socorro dove (Mexico), Père David's deer (China) and European bison

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## Why captive breed?

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Many species are incapable of surviving in their natural habitats due, predominantly, to human impacts

For terrestrial vertebrates alone, it is estimated that 4000–6000 species will require captive breeding over the next 200 years to prevent extinction, the number having doubled recently due to the amphibian crisis (Soulé *et al.* 1986; Tudge 1995; Anonymous 2006; Mendelson *et al.* 2006). At least 25 animal species, including addax, Arabian oryx, black-footed ferret, California condor, greater stick-nest rat, mala, Père David's deer, Przewalski's horse, scimitar-horned oryx and 11 species of *Partula* snail, plus several plants, including the Franklin tree and Cook's kok'io, have been preserved in captivity following extinction in the wild. Further, many threatened species have captive populations that act as insurance against extinction in the wild. Most managers of zoos, botanic gardens and wildlife parks accept the necessity to:

- retain species as dynamic evolutionary entities
- maintain the genetic 'health' of species for long-term viability
- retain the option of subsequent release back to natural habitats.

IUCN, the premier world conservation body, has endorsed captive

breeding as an essential component in the conservation of species

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Captive breeding programs assist conservation by (IUCN [1987](#)):

- establishing populations in secure *ex situ* locations
- educating and engaging the public on conservation issues
- providing a focus for fund-raising efforts for conservation
- providing animals for research on the basic biology of species, knowledge that can then be applied to conservation of species in the wild
- providing animals for reintroduction programs, where applicable.

***Ex situ*** conservation procedures range from relatively simple cultivation of plants and husbandry of animals to high-technology procedures such as artificial insemination and cryopreservation of organisms, cells and gametes. These are being applied to a wide variety of animals and plants, especially the more charismatic species.

## **Extent of captive breeding and propagation activity**

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Considerable resources are being devoted to captive breeding and other *ex situ* conservation activities, but they are inadequate to conserve all needy threatened species

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As of 2007, 987 threatened and near-threatened vertebrate species were being captive bred (L. Bingaman Lackey, pers. comm.), a four-fold increase

since 1989/90 (Magin *et al.* [1994](#)). This contrasts sadly with the requirement to captive breed approximately 4000–6000 species of terrestrial vertebrates alone. In total, the International Species Information System (ISIS: [www.isis.org](http://www.isis.org)) has records on almost 112 000 individuals of threatened and near-threatened species.

The Royal Botanic Gardens at Kew, England houses about 2700 out of ~25 000 threatened species ([www.rbgekew.org.uk](http://www.rbgekew.org.uk); Primack [2006](#)). The Center for Plant Conservation, USA, now maintains >600 endangered US plant species. Overall, about 30% of all vascular plants are represented in the collections of ~1500 botanic gardens worldwide (Briggs & Walters [1997](#)).

## Zoos in the twenty-first century

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The menageries of the nineteenth century are now zoological parks and the best are major animal conservation centres

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Many zoos have transformed from consumers of wildlife to become important conservation institutions. The menageries of the late nineteenth and early twentieth centuries collected wildlife from nature and exhibited them, primarily as just one or two individuals. Zoo collections changed dramatically in the 1970s (Ginsberg [1993](#)). Wildlife populations were declining, legislation was being adopted to limit the trade in endangered species (e.g., US Endangered Species Act in 1972; CITES in 1973), and a conservation ethic was developing. The late Gerald Durrell, engaging author and founder of the Jersey Wildlife Preservation Trust and Jersey Zoo, was a leading figure in promoting the role of zoos in conservation. They began to maintain larger collections and to devote more effort to breeding of endangered species in captivity.

The World Association of Zoos and Aquariums (WAZA: [www.waza.org](http://www.waza.org)) is a global organization encompassing ~1300 zoos and aquariums, which receive over 600 million visitors annually. In 2005 WAZA reaffirmed their conservation role (WAZA 2005). The World Zoo and Aquarium Conservation Strategy sets out the policies and standards to be reached by all zoos and aquariums in population management, education, research, public relations and animal welfare for biodiversity conservation. Perhaps 5–10% of the available spaces could be used for endangered species (Seal 1991).

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Zoos are now involved in a wide range of collaborative captive breeding programs and in general conservation activities

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By the early 1980s, zoo managers recognized that their contribution to conservation could best be made through regional and international cooperative breeding programs, as they needed to avoid problems arising from inbreeding. In North America, Species Survival Plans (SSPs), co-ordinated by the Association of Zoos and Aquariums, were first developed in 1981. SSPs involve co-ordinated management of all captive individuals held by cooperating institutions, and are now in place for 161 species, most of which are threatened (AZA 2007). **Studbooks** (computer databases containing the pedigree and life history of all individuals in the population) are used in devising recommendations on which animals should breed, with whom, how often and where. There are about 1150 regional and 182 international studbooks for endangered and rare species, the latter being managed by WAZA. Breeding animals are frequently moved among participating institutions to optimize genetic benefit. Similar cooperative programs have been developed in many other countries and regions.

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ISIS provides a central repository of information on captive animals

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Pedigrees, individual histories, breeding experiences and health records are collected at each zoo and maintained by ISIS in Minnesota. ISIS has information on 2 million animals from 10 000 species from 650 zoos and wildlife parks in over 70 countries (ISIS 2007). ISIS has developed record keeping software such as the Animal Record Keeping System (ARKS), Single Population Analysis and Record Keeping System (SPARKS) and Medical Animal Record Keeping System (MEDARKS), and is now developing Zoological Information Management System (ZIMS), a global, real-time web-based application that pools information across all aspects of animal management.

Zoos also contribute to both *ex situ* and *in situ* conservation, through involvement with the Conservation Breeding Specialist Group (CBSG) of IUCN, led by Robert Lacy. Their programs include (a) Conservation Assessment and Management Plans (CAMPs) that provide initial assessments of the global status of a species and initial research and management recommendations, (b) population and habitat viability assessments (PHVA) that estimate extinction risk, evaluate management options and make recommendations for conservation action (Chapter 22), (c) zoo conservation planning, (d) providing expertise relating to captive breeding and (e) co-ordinating meetings and workshops on topics required to advance conservation, etc. CBSG has also facilitated resolution of disputes among agencies and groups involved in high-profile conservation programs (e.g. for Florida panthers, black-footed ferrets and giant pandas).

Captive populations can provide animals for reintroduction programs, as discussed in the next chapter.

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A long-term goal for many captive breeding programs is to eventually reintroduce species back into the wild

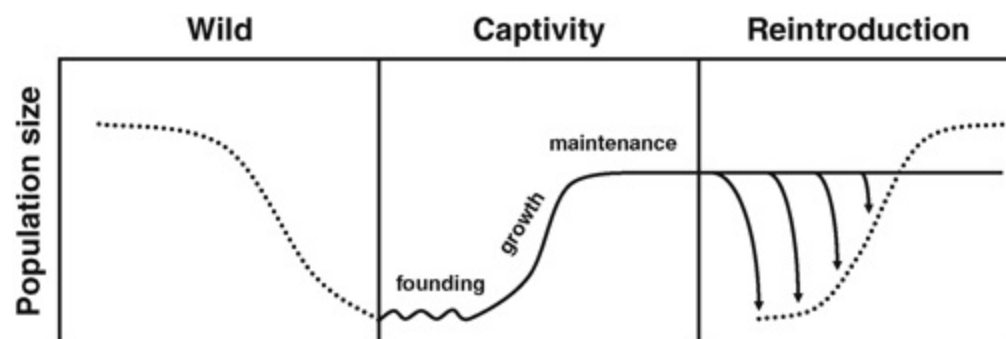
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## Stages in captive breeding and reintroduction

Captive breeding and reintroduction may be viewed as a process involving up to six stages (Fig. 19.1):

- (1) Recognizing decline of the wild population and its genetic consequences
- (2) Founding one or more captive populations
- (3) Expanding the captive populations to a secure size
- (4) Managing the captive population over generations
- (5) Choosing individuals for reintroduction
- (6) Managing the reintroduced population (probably fragmented) in the wild.



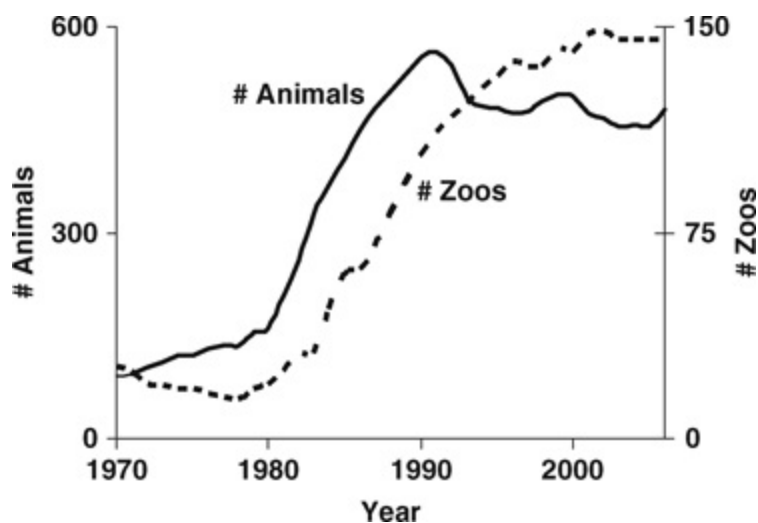
**Fig. 19.1** Captive breeding and reintroduction as components of a six-stage process.

Essential genetic issues in the first stage are the rate of decline of the wild

population, the size to which it is reduced, and the resulting loss of genetic diversity and increased inbreeding prior to captive breeding. The first four issues were introduced in [Chapters 11–15](#) and their management implications are discussed here. Additional aspects of the fourth issue, and genetic management of reintroductions, are deferred until the next chapter.

Management during the foundation, growth and maintenance phases focuses on different priorities. During foundation, population size is usually small, and knowledge of the husbandry of the species is often lacking. Management concentrates on basic research to develop husbandry techniques and efforts to ensure reproduction of the founders.

During the growth phase, the goal is rapid reproduction and dispersion of the population to multiple facilities. During the maintenance stage, the population is managed at a predetermined target size. Typically, animals are not removed from the captive population (e.g. for reintroduction) until the population size approaches this target size. Growth of the captive population of golden lion tamarins over these three phases is illustrated in [Fig. 19.2](#).



**Fig. 19.2** Growth of the golden lion tamarin captive population (J. D. Ballou & J. Mickelberg, unpublished data). Foundation occurred between 1970 and 1980, growth from 1980 to 1990 and the population has been stably maintained with intense genetic management from 1990 to 2007.



## Founding captive populations

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Captive populations should be founded from at least 20–30 contributing breeding individuals of known source. However, many captive populations of endangered species have been founded with fewer individuals, with considerable loss of genetic diversity and inbreeding

A fully representative sample of founders is required, if the population is to encompass the genetic diversity in the wild and minimize subsequent inbreeding. In practice, founders often derive from different sources or are of unknown origin. Similarly, most captive populations have been established using inadequate founder numbers. Some captive populations were only initiated when the endangered species was ‘at the last gasp’, with few founders available. Founders for many other captive breeding programs were the few animals that were already in captivity at the time the program was formalized ([Table 19.1](#)). Several of the founders of the Asiatic lion program were later identified as African × Asiatic lion hybrids, resulting in termination of the program, after substantial resources had been expended.

**Table 19.1** Founder numbers in captive populations of endangered species. *Many species have fewer than the recommended minimum of 20–30 founders*

Species	Number of founders
Mammals	
Arabian oryx	10
Black-footed ferret	10
European bison	13
Golden lion tamarin	48
Indian rhinoceroses	17
Przewalski's horse	12 (+ 2 or more domestic horses)
Red ruffed lemur	7
Siberian tiger	25
Snow leopard	7
Speke's gazelle	4 (1♂, 3♀)
Birds	
California condor	14 (3 clans)
Guam rail	9
Lord Howe Island woodhen	6 (3 pairs)
Mauritius pink pigeon	6
Plain pigeon	21
Puerto Rican parrot	13
Invertebrates	
British field cricket	12 (6 pairs)

Sources: Ryder (1988); Brock & White (1992); Hedrick & Miller (1992); Haig *et al.* (1994); Olney *et al.* (1994); Tongue (1999).



Arabian oryx



Lord Howe Island woodhen

Typically, only a moderate proportion of wild-caught individuals successfully breed in captivity. For example, 242 wild-caught golden lion tamarins entered the captive population, but only 48 contributed to the current gene pool, and two-thirds of the gene pool prior to management was derived from just one prolific pair ([Box 20.1](#)). To account for this, the number of individuals brought into captivity needs to be  $\gg 20\text{--}30$  to meet the target of 20–30 contributing founders.

There are economic trade-offs among the number of founders, the cost of starting captive populations and the subsequent size required to maintain 90% of genetic diversity for 100 years ([Chapter 15](#)). With few founders, the initial cost of obtaining individuals is minimized, but large populations are required to maintain genetic variation and avoid inbreeding, and subsequent costs will be much greater. With larger founder numbers, the initial costs are higher, but subsequent costs are reduced, yielding substantial savings in the long term (Mansour & Ballou [1994](#)). The need to acquire a representative genetic base, the low proportion of founders that reproduce, and economics all argue for establishing populations with as large a number of founders as possible.

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IUCN recommends that captive populations be established when wild population numbers fall below 1000

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To avoid detrimental genetic impacts, captive populations should be established before the wild population approaches extinction. Consequently, IUCN (1987) recommended that captive populations be founded before wild populations drop below 1000 individuals. Benefits of this strategy include (1) the ability to obtain wild founders with low inbreeding levels, (2) reduced impact of removal on wild populations, and (3) time is allowed to develop suitable husbandry techniques.

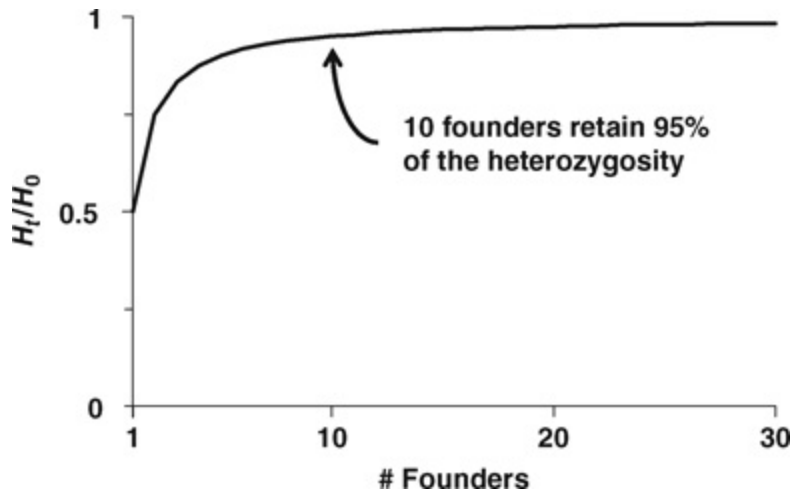
## Genetic consequences of small founder numbers

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Small founder numbers lead to loss of genetic diversity, rapid rates of inbreeding and inevitable inbreeding depression

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Only three of the captive populations referred to in [Table 19.1](#) had founders approximating the recommended minimum of 20–30 contributing founders. Most will have lost genetic diversity and become inbred more rapidly than desirable. The proportion of heterozygosity retained is  $[1 - 1 / (2N_e)]$  ([Equation 8.2](#)), as illustrated in [Fig. 19.3](#). Thus, even 10 contributing founders capture 95% of the heterozygosity in an outbreeding species, while 30 founders capture over 98%.



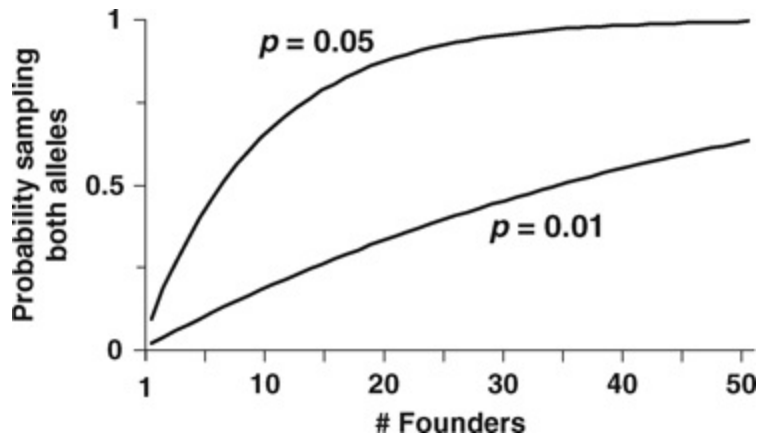
**Fig. 19.3** Relationship between the number of founders and the proportion of the heterozygosity that they capture. *20–30 contributing founders will capture most of the heterozygosity in the source population.*

The number of founders required to capture allelic diversity depends on the number and frequencies of alleles in the source population, but typically requires more individuals than needed to capture heterozygosity, especially if rare alleles are to be sampled. Marshall & Brown (1975) recommended that the number of founders be sufficient to obtain, with a 95% certainty, all the alleles with a frequency greater than 0.05 at a random locus in the target population.

For a locus with two alleles, the probability that a random sample of  $n$  founders contains at least one copy of each allele  $P(A_1, A_2)$  is given by:

$$P(A_1, A_2) = 1 - (1 - p)^{2n} - (1 - q)^{2n} \quad (19.1)$$

where  $p$  is the frequency of allele  $A_1$  and  $q$  is the frequency of  $A_2$ .  $P(A_1, A_2)$  is plotted against founder number in Fig. 19.4 for loci with allele frequencies at (0.95, 0.05) and (0.99, 0.01). At least 30 founders are needed to meet Marshall and Brown's recommendations. For alleles at a frequency of 1%, even 50 founders have only ~60% chance of capturing the allele. Rare alleles are unlikely to be sampled unless founder numbers are very high.



**Fig. 19.4** Probability of sampling at least one copy of each allele against number of founders for loci with alleles at frequencies of  $p = 0.05$  and  $p = 0.01$ .

## Genetic background of the founders

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The taxonomic identity of founders should be well defined

A critical consideration in founding captive populations is the source of the founders. Unwittingly crossing different sub-species or species has decreased the conservation value of many captive breeding programs, including those for orangutans, giraffes, spider monkeys, gibbons, lions, tigers and lesser white-fronted geese (Ruokonen *et al.* 2007). Crossing different sub-species or species also increases the likelihood of outbreeding depression, although there is little evidence that this is a major problem in captive breeding programs (Lacy *et al.* 1993; Ballou 1995; Chapter 16).

## Growth of captive populations

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During the growth phase for captive populations, priority is on rapid population growth as opposed to intense genetic management

Rapid expansion creates a demographically secure population. Attempts are made to breed from most adults, not just those genetically most valuable. Genetic management during this phase is limited to avoiding pairing of close relatives, ensuring that all animals have the opportunity to breed, and that the genetically most valuable animals are placed in viable breeding situations.

Limited genetic management was applied during the growth phase in golden lion tamarins, black-footed ferrets and California condors (still growing), but subsequently these programs have been subjected to intensive genetic management, as detailed below.

## Defining the target population size

The target size is the population size required to retain 90% of genetic diversity for 100 years

The required number of animals to retain 90% of genetic diversity for 100 years depends critically on **founder effects**,  $N_e/N$  ratios and generation lengths, and on how quickly the population increases after the founding bottleneck ([Chapter 15](#)). For example, a small founder population will require a larger  $N_e$  overall to compensate for genetic loss during the bottleneck. The

$N_e/N$  ratio depends on variance in family size, sex-ratio, mating system and fluctuations in  $N$  ([Chapter 11](#)).

## **Genetic management during the maintenance phase**

### **Genetic deterioration in captivity**

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Captive populations deteriorate genetically through inbreeding depression, loss of genetic diversity, and genetic adaptations to captivity that are deleterious in the wild

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As the population approaches its target size, management increasingly shifts to genetic issues. The objective becomes maintaining demographic stability and counteracting deleterious genetic changes. As discussed previously, the effects of inbreeding depression, loss of genetic diversity and mutational accumulation are all more severe in smaller than in larger populations. Conversely, genetic adaptation to captivity is greater in larger than in smaller populations. While this is beneficial in captivity, it is overwhelmingly deleterious when captive populations are returned to the wild (Frankham [2008](#)).

### **Inbreeding and inbreeding depression**

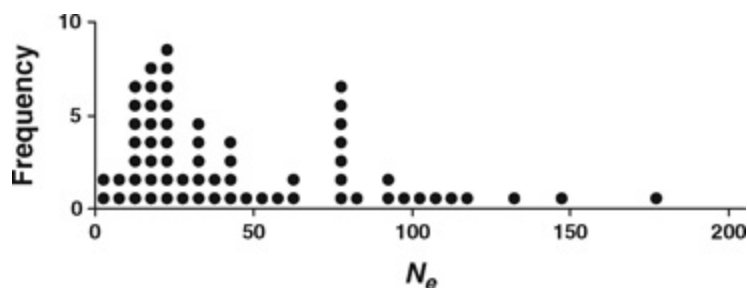
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Many captive populations of threatened species are too small to avoid inbreeding depression within a few generations



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Effective sizes of captive populations for endangered species in SSP programs are given in Fig. 19.5. Most of these are so low that they suffer inbreeding depression and significant loss of genetic diversity over relatively few generations. For example, inbreeding coefficients are about 20% in Przewalski's horse (Box 12.1), 30% in the South China tiger and reached 61% in zoo-bred Mexican wolves. To minimize such problems, the total captive population of an endangered species is often managed as a single unit with interchange among zoos, under regional (e.g. SSPs), or global management plans (e.g. golden lion tamarin).



**Fig. 19.5** Distribution of effective population sizes in SSP programs for endangered species (from Hodskins 1997). This assumes an  $N_e/N$  ratio of 0.3.

Despite interchange of individuals or gametes, these pooled populations are still not large. The SSP programs have a mean  $N_e$  of only 41, small enough that inbreeding depression due to finite size will occur (Chapter 13). At such a size, populations will develop an inbreeding coefficient of  $F = 0.26$  after 25 generations, greater than that arising from one generation of full-sib mating.

## Loss of genetic diversity

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Captive populations lose genetic diversity at foundation, and as a result of subsequent small population sizes

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The effect of founder numbers, versus subsequent population size, on loss of genetic diversity can be determined by recasting [Equation 11.1](#), as follows:

$$\frac{H_t}{H_0} = \left(1 - \frac{1}{2N_{fo}}\right) \left(1 - \frac{1}{2N(N_e/N)}\right)^{t-1} \quad (19.2)$$

where  $N_{fo}$  = number of effective founders. The first term reflects the founder effect and the second term is the effect of subsequent population size restrictions. From [Equation 19.2](#), retention of genetic variation in captive breeding programs can be maximized by:

- maximizing the initial genetic variation by using adequate numbers of founders
- minimizing the number of generations in captivity by seed storage in plants, cryopreservation or breeding from older animals
- maximizing population size
- maximizing  $N_e/N$ .

In the long term, the major contribution to loss of genetic diversity is typically the subsequent restriction in effective population size, rather than the size at foundation.

In captive populations of endangered species,  $N_e/N$  ratios of 0.2–0.4 are typical (Mace [1986](#)). This is lower than the theoretical maximum of 2.0, but better than in unmanaged populations where 0.1 is typical. While there is still considerable room for improvement, practical considerations (mate incompatibility, compliance with veterinary and other management recommendations, and stochastic factors) will probably always limit maximization of  $N_e/N$ .

## **Current genetic management of captive populations**

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Captive populations are managed to minimize genetic changes in captivity

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Since the founders are presumed to be a representative sample of the wild population (frequently not the case), this goal translates into minimizing any changes to the founder gene pool from one generation to the next. In other words the aim is to ‘freeze’ evolution in captive populations. As this is never possible, the recurrent goal of genetic management translates into accepting a loss of genetic diversity, and a concomitant increase in inbreeding coefficient, of no more than 10% over 100 years (see [Chapter 15](#)). Unfortunately, such goals are unattainable in many populations (due to small founder numbers, and/or limitations in space), and the genetic objective is often relaxed (e.g. 80% for 100 years, or 90% for 50 years). If reintroduction appears feasible, the length of a program may be shortened. It is disturbing that there has been a continuing relaxation of goals since they were first defined. The original proposal was to retain 90% of genetic diversity for 200 years (Soulé *et al.* [1986](#)), but goals as low as 80% for 50 years are now appearing.

Whilst a range of genetic strategies have been proposed and used for maintaining genetic diversity during captive management, minimizing mean kinship is now preferred and widely used.

## **Minimizing mean kinship**

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The recommended genetic management regime is to choose parents to minimize mean kinship

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Managing populations by minimizing mean kinship is an effective way to deal with unequal founder contributions, complex pedigrees and prior inbreeding. In brief, minimization of kinship chooses individuals with least relationship to the population as parents of the subsequent generation. It reduces inequalities in founder contributions. In a population with unrelated founders, it is the same as equalization of family sizes. It is also very similar to maximum avoidance of inbreeding (see below).

Using both theoretical predictions and computer simulation Ballou & Lacy (1995) showed that management by minimizing mean kinship was the best of several strategies for retaining genetic diversity. In experiments with fruit flies, populations managed by minimizing kinship retained more genetic diversity than those managed with maximum avoidance of inbreeding, or unmanaged populations (Montgomery *et al.* 1997). However, there were no significant differences among treatments in reproductive fitness.

We now define kinship, show how it is calculated, define mean kinship and provide an example of its use.

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The kinship of two individuals is the inbreeding coefficient of their theoretical offspring

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The **kinship** ( $k_{ij}$ , or **coancestry**) of two individuals  $i$  and  $j$  is formally defined as the probability that two alleles taken randomly, one from each,

will be identical by descent. Since this is also the definition of inbreeding ([Chapter 12](#)), the kinship between two individuals is the same as the inbreeding coefficient of their (hypothetical) offspring.

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The mean kinship for individual  $i$  ( $mk_i$ ) is the average of kinship values for that individual with every individual in the population, including itself

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The **mean kinship** is:

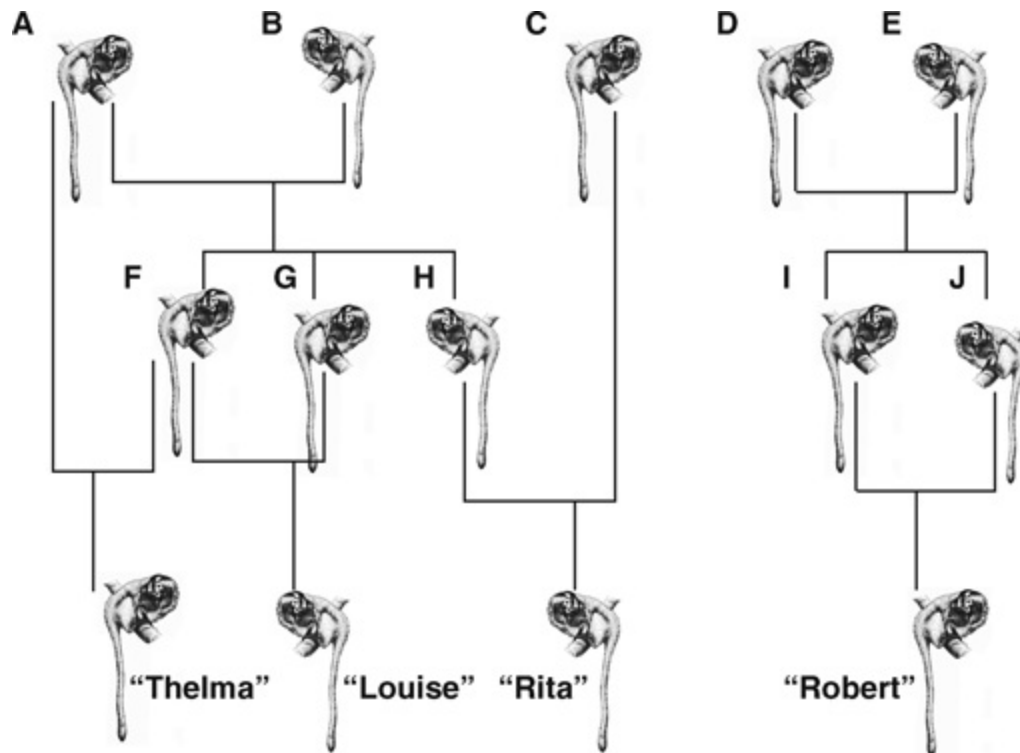
$$mk_i = \frac{\sum_{j=1}^N k_{ij}}{N} \quad (19.3)$$

where  $k_{ij}$  = kinship between  $i$  and  $j$ , and  $N$  is the number of individuals in the population. Example 19.1 illustrates the computation of mean kinship.

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### **Example 19.1 Computation of mean kinships**

The kinships for the named individuals in this pedigree are given in the table below. Mean kinships are the averages of kinships across the row.

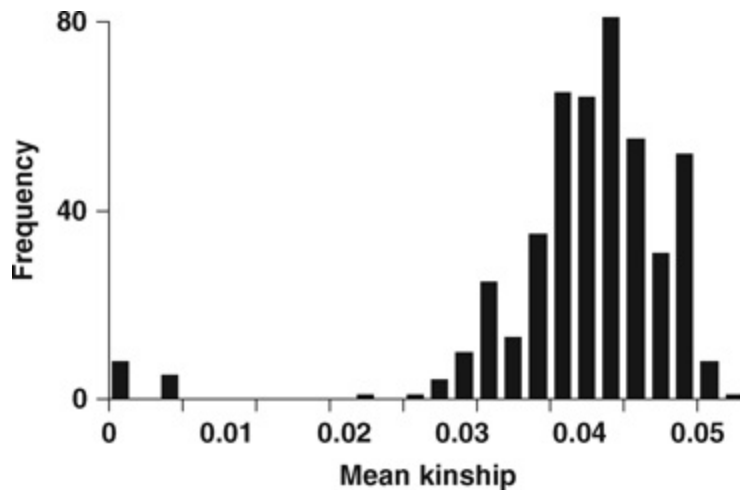


	Thelma	Louise	Rita	Robert	Mean kinship
Thelma	5/8	5/16	1/8	0	0.266
Louise	5/16	5/8	1/8	0	0.266
Rita	1/8	1/8	1/2	0	0.187
Robert	0	0	0	5/8	0.156

Thus, the mean kinship for Thelma is  $(5/8 + 5/16 + 1/8 + 0) / 4 = 0.266$ .

Note that all animals, except Rita, are inbred, and their kinships with themselves are therefore greater than 0.5.

The distribution of mean kinship in the entire population of 480 golden lion tamarins is given in the [Fig. 19.6](#) below.



**Fig. 19.6** Distribution of mean kinship values of 480 golden lion tamarins. The individuals with the lowest mean kinships are recently acquired founders and  $F_1$  descendents of founders in Brazilian zoos that have yet to reproduce.

The average mean kinship for a population is the expected inbreeding coefficient in the next generation with random mating. Consequently, the average mean kinship is inversely related to genetic diversity ( $H_t/H_0$ ), as follows:

$$\overline{mk} = \bar{F}_{t+1} = 1 - \frac{H_{t+1}}{H_0} \quad (19.4)$$

Consequently, *if kinship is minimized, heterozygosity is maximized*. It is also highly effective in conserving allelic diversity (Fernández *et al.* 2004).

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Mean kinship for a population is inversely related to genetic diversity

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Individuals with low mean kinship are the most valuable. They have fewer

relatives in the population than individuals with high mean kinship and therefore carry fewer common alleles. For the pedigree in Example 19.1, Robert has the lowest mean kinship (0.156) and he is the most valuable animal in his generation as he is unrelated to Rita, Louise and Thelma. Conversely, Thelma and Louise share most genes with the rest of the population, have the highest mean kinships (0.266) and are least valuable genetically. Under a breeding program to minimize mean kinship, animals with lower mean kinship are given breeding priority. In this example we would increase the contribution of genes from Robert and decrease those of Thelma and Louise.

When all animals are related with the same kinship, all individuals have the same mean kinship and managing by mean kinship becomes equalization of family sizes, which also approximately doubles the effective population size ([Chapter 11](#)).

## **Applying mean kinship breeding strategies**

When applying minimizing kinship in a breeding program, those individuals with the lowest mean kinships are chosen to be parents. Two additional considerations are required to determine specific matings. First, matings between individuals with markedly different mean kinships are avoided as they limit future management options. For example, if a valuable individual is mated to one of low value, increasing the contribution of the under-represented individual also increases the contribution of its over-represented mate. For example, the two giant pandas at the National Zoo, in Washington, DC, have very high and very low mean kinship values. Therefore, it has been decided to mate the valuable female with a valuable male from San Diego Zoo using artificial insemination. Second, mating of close relatives is avoided to minimize inbreeding. For example, two sibs with low mean kinships should not be mated to each other. In complex pedigrees, mean kinship and inbreeding are computed using software, such as SPARKS (Scobie [1997](#)) in combination with PMx (Pollak *et al.* [2009](#)).



Procedures to formally cope with avoidance of matings between close relatives, whilst minimizing mean kinship, have been considered by Fernández *et al.* (2001). Where pedigree information is unavailable, relatedness can be inferred from molecular markers, such as microsatellites and AFLPs (Fernández & Toro [2006](#); Dasmahapatra *et al.* [2008](#)).

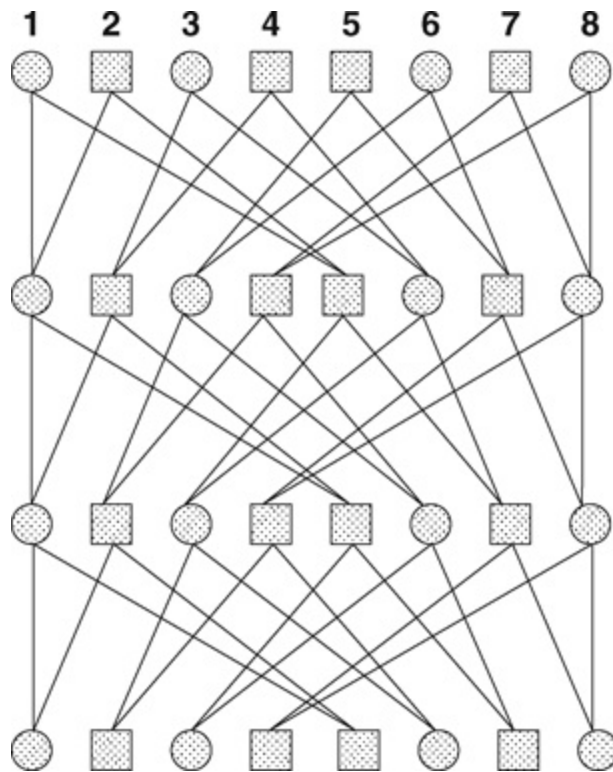
## Maximum avoidance of inbreeding

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Maximum avoidance of inbreeding schemes involve equalization of family sizes and a circular mating pattern

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A maximum avoidance of inbreeding scheme is shown in [Fig. 19.7](#). This scheme involves equalization of family sizes and delays inbreeding for as long as possible using a circular mating system (Crow & Kimura [1970](#)).



**Fig. 19.7** Maximum avoidance of inbreeding.

The example has eight unrelated founders, and female 1 mates with male 2, female 3 with male 4, etc. Each of the four matings produces one female and one male. In the next generation, the female from  $1 \times 2$  mates to the male from  $3 \times 4$ , the female from  $5 \times 6$  mates with the male from  $7 \times 8$ , etc. In the succeeding generation, the female from  $(1 \times 2) \times (3 \times 4)$  mates with the male from  $(5 \times 6) \times (7 \times 8)$ . After this point, inbreeding cannot be avoided. To be fully effective, however, the scheme only works when applied from the first (founder) generation and it requires that equal numbers of breeding females and males are produced to become parents of the next generation.

Unfortunately, this scheme is very difficult to apply to captive populations of threatened species. First, genetic management generally begins with individuals several generations removed from the founders. Second, the scheme must be followed precisely to be effective, with no practical allowance for mortality of offspring and parents, incompatibility of breeders, reduced fertility, etc. Captive breeding programs are frequently confronted with complex pedigrees with unequal founder representation, high levels of

inbreeding and loss of genetic diversity.

## **Incomplete pedigree information**

The minimizing mean kinship protocol requires that the pedigree of the population is completely known and all founders are unrelated. However, in many captive populations, ancestry is not known for all individuals. Molecular genetic techniques can be used to determine parentage when putative mates and offspring are available for sampling ([Chapter 21](#)), but it is often not possible to resolve unknown ancestry many generations in the past. Methods have been developed to estimate mean kinship when part of the parentage is unknown, but this does not provide optimal management of genetic diversity (Ballou & Lacy [1995](#)).

Except for very rare cases, the relationships among founders are not known and, if the founders themselves are derived from a small population, it is likely that they are related. However, molecular markers such as microsatellites can be used to estimate levels of kinship among founders. This has been utilized for captive breeding programs of the California condor, Guam rail, and several other species ([Chapter 21](#)).

## **Limitations of management by minimizing kinship**

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Minimizing kinship in a single large population does not address all the genetic concerns in captive breeding. However, its use in partially fragmented populations is likely to be optimal

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While minimizing kinship maximizes retention of genetic diversity in a single population, it is not the only defensible means for genetically

managing captive populations. Management of all captive animals of a species as a single large unit involves frequent movement of animals among institutions, entailing high costs and risks of contagion. Further, loss of genetic variation is least in populations maintained as sub-populations, rather than as a single population, provided none of the sub-populations suffers extinction ([Chapter 14](#)). A partially fragmented population structure (with each fragment managed using minimising kinship) will also reduce genetic adaptation to captivity, an important consideration if the species is destined for reintroduction ([Chapter 20](#)). Such management is considered in the next chapter.

## Captive management of groups

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Where pedigrees are unavailable, management of groups by maximum avoidance of group inbreeding is recommended

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Some species breed best in multi-female, multi-male groups, where paternities are unknown. For example, chimpanzees and many ungulates breed best in groups, while other species live in colonies (social birds and mammals, many fish and corals). For these species minimizing mean kinship based on pedigrees cannot be used, and management may be based on either management for groups (below), or on kinship inferred from multilocus genotypes. Inferred kinship is inferior to pedigree-based kinship, but it will often be better than treating all individuals as equally valuable genetically (Oliehoek *et al.* [2006](#)).

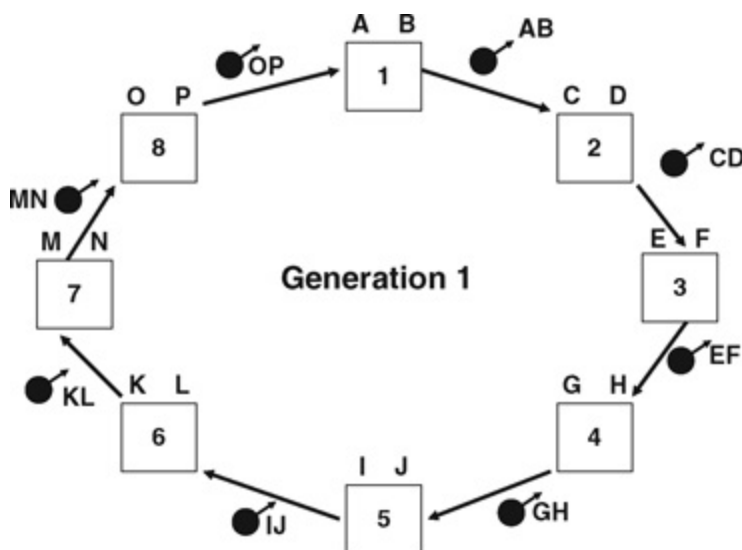
A group breeding strategy is illustrated in [Box 19.1](#). This procedure utilizes the maximum avoidance of inbreeding (MAI) scheme described above ([Fig.](#)

19.7), but applied to groups rather than to individuals. Contributions of groups to be parents of the following generation are equalized and individuals are regularly exchanged among groups. In the first generation in [Box 19.1](#) males are moved one group in a clockwise direction. In the second generation, males are moved two groups in a clockwise direction. In this example, inbreeding cannot be avoided after the third generation.

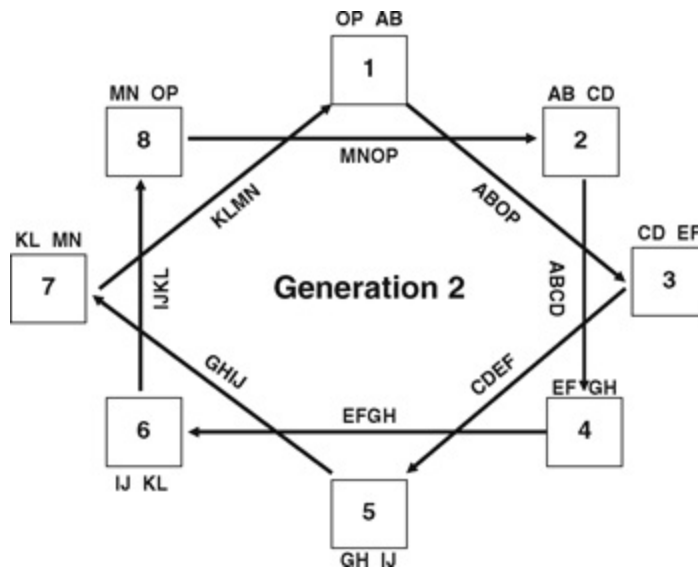
### **Box 19.1 Low-intensity genetic management for groups using maximum avoidance of inbreeding (Princée 1995)**

Application of maximum avoidance of inbreeding to groups is illustrated with eight groups in the two figures below.

Maternal and paternal lineages are indicated with letters AB, CD, . . . , OP. Boxes represent breeding groups. Arrows indicate transfer of males from natal groups to host groups. Bloodlines of males are shown near the arrows. The operation of the MAI system can be illustrated by following group 1. In generation one, males are moved to the next group in a clockwise direction. Group 1 starts with lines A and B. Males of group 1 (bloodlines AB) are moved to group 2. Male offspring of group 8 (bloodlines OP) are moved into group 1.



In the second generation, male offspring are moved two groups in a clockwise direction, as moving males by one group would result in inbreeding. Moving males by two groups results in group 1 males (ABOP) being mated to group 3 females (CDEF), thus avoiding inbreeding, i.e. no original breeding group is genetically represented in both males and females.



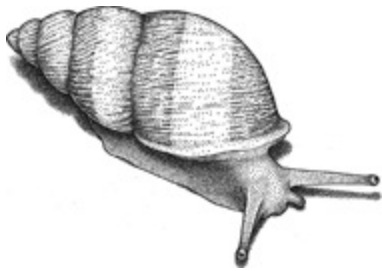
In the third generation, males are moved four groups in a clockwise direction, 1 to 5, 2 to 6, 3 to 7 and 4 to 8, again avoiding inbreeding. However, inbreeding can no longer be avoided after the third generation as all groups have bloodlines ABCDEFGHIJKLMNOP. Exchange of individuals in the fourth generation reverts to that in generation one and the cycle is repeated.

Detailed guidelines for applying this system to different numbers of groups are provided by Princée (1995).

Group genetic management is also an option that can be used for non-threatened species, as it is a less intensive procedure when resources are insufficient for full scale genetic management by minimizing kinship. It is also suitable for management of small wild populations, but the form shown

in [Box 19.1](#), with the movement of all males, would be expensive.

Group management is being applied with *Partula* snails, but the procedures are an advance on those above. They account for differences in group size and prior gene flow in a manner analogous to mean kinship strategies for individuals. Using information on transfers of individuals between groups, splits and merging of groups, observations on births and deaths within groups, as well as the breeding systems within groups (e.g., sexual, selfing or clonal), it is possible to estimate levels of genetic kinships among populations and manage to minimize it (Wang [2004c](#)).



Partula snail

## Managing for adaptive genetic diversity

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There is currently insufficient information to manage populations for specific conservation of adaptive genetic diversity, rather than total genetic diversity

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The schemes above are all based on maximizing total genomic diversity. However, only some of this will be of adaptive significance in the current environment. Why do we not manage to maximize the adaptive component of genetic diversity? Whilst this is highly desirable, we cannot identify most of the adaptive loci in most threatened species. Further, loci that have adaptive

genetic diversity in the current environment may differ from those that are required to adapt to future environments. The prospects for managing individual-locus genetic diversity and the risks of such approaches were discussed in [Chapter 10](#).

## Ex situ conservation of plants

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Most ex situ conservation of plants is done by seed storage with occasional captive propagation to regenerate seed. Thus, the emphasis is on collecting germplasm to maximize genetic diversity

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Genetic management of captive plant populations is not nearly as complex as in animals. Many plants can be stored as seeds, and some can be cryopreserved. They therefore progress through fewer generations in captivity and suffer little inbreeding, loss of genetic diversity or genetic adaptation due to captive propagation. Consequently, the major issues for most plants are the sampling regimes used in collection of specimens for captive propagation or storage (Frankel *et al.* [1995](#)). However, preservation of seeds is not possible for about 15% of, mainly tropical, species that lack seed dormancy (Briggs & Walters [1997](#)). In these plants, genetic management has to address the same issues as in animals.

Two features of sampling are the size of samples and ensuring that collected material is representative and encompasses the full genetic diversity of the species. Representation is a greater concern for plants than for animals, especially if the species is selfing. Here, a higher proportion of genetic diversity is distributed among rather than within populations, particularly for adaptive genetic variation ([Chapters 3 and 17](#)).



Generally, recommended sampling regimes involve collecting 1–20 seeds per individual from each of 10–50 individuals from each of five separate threatened populations (Falk & Holsinger [1991](#)). Such sampling would also be desirable in founding captive populations of animals but, in practice, the luxury of time or resources is not available.

The emphasis in plants was initially on conservation of species of commercial value, especially domesticated plants and their wild relatives but, endangered plants are receiving increasing conservation effort, as described earlier in this chapter (Falk & Holsinger [1991](#); Bowles & Whelan [1994](#); Falk *et al.* [1996](#)).

## Reproductive technology and genome resource banks

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Reproductive technology and genome resource banking can contribute significantly to genetic management, but have only limited current use

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Reproductive technologies, such as artificial insemination, cryopreservation and various cloning techniques, promise to have significant input to conservation of threatened species. The use of these techniques in endangered species is currently very limited and is usually only successful in species closely related to domestic animals (Pukazhenthil *et al.* [2006](#)). Almost all of these techniques require extensive research, as the required conditions are species specific, rather than broadly applicable.

## Artificial insemination

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Artificial insemination is potentially a useful tool in conservation and works in a growing number of species, but is currently used routinely in only a handful of species

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Artificial insemination (AI) can be used to capture the genetic contribution of individuals that are unable to breed naturally, and is being used in black-footed ferrets, cheetahs, giant pandas and whooping cranes.

Artificial insemination with frozen semen can be used to transfer genetic material between populations with great reductions in cost. It can also be used to reduce distortions in sex-ratios of breeders by inseminating females with semen from males other than the local dominant, or sole, male.

To our knowledge, artificial insemination is only being used routinely in the threatened species mentioned above, and it is commencing use in elephants.

## **Cryopreservation**

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Cryopreservation is potentially a very valuable tool in conservation, but currently works for only a small minority of animals related to domestic species. It is applicable to a much broader range of threatened plant taxa

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Cryopreservation of sperm or embryos in genome resource banks provides a valuable means for extending the generation interval, thereby slowing inbreeding and loss of genetic diversity (Johnston & Lacy [1995](#)).

Cryopreserved animal cells, embryos and gametes can provide genetic material for future use, as indicated above. Animal cells provide the potential for regenerating individuals in the future via cloning, in a similar manner to tissue culture of plant cells. Cryopreserved gametes and embryos parallel seed banks in plants. However, in animals an adult population must be maintained, so that mature females are available to inseminate, or to raise embryos. In a few cases, related domestic species can act as surrogate mothers. This has been done with an African wildcat and Indian desert cat (into domestic cats), gaur (into cattle) and mouflon sheep (into domestic sheep) (Lanza *et al.* [2000](#)).

Cryopreservation is currently only applicable to a small proportion of threatened animals, as the technology needs to be customized for each species. Cryopreservation appears more generally applicable to plants, and has more immediate value to a wide range of species. For example, seed from 68 of 90 native Western Australian plant taxa germinated after storage in liquid nitrogen (Touchell & Dixon [1993](#)).

Gene banking of purified DNA serves as an invaluable source of material for research, but is most unlikely to contribute to living animal populations (Ryder *et al.* [2000](#)). Several DNA banks are already in existence. They will in the future allow tests to determine whether genetic diversity has been lost, in a manner similar to studies using museum specimens.

## Cloning

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Cloning can aid in conservation. It is widely available in plants, but currently only works in a very small proportion of threatened animals

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Cloning is easily achieved via cuttings or tissue culture in plants, where many copies of each individual can be made. However, many genotypes must be cloned if it is to be used to preserve genetic diversity. Clones have been made from all known wild individuals of Wollemi pine in Australia (Woodford [2000](#)).

In animals, nuclear transplantation has been used to clone a variety of domesticated or laboratory mammals, amphibians and fish, as well as the endangered gaur, Banteng, African wildcat and bucardo (Holt *et al.* [2004](#); Westphal [2004](#)). In each case, eggs from an abundant domestic animal (cow, cow, domestic cat and goat, respectively) were harvested, their nuclei replaced with those from the endangered species and the resulting embryos raised in the domestic species. To date the success rate of nuclear transplantation is very low, with only a few live births from hundreds of implanted embryos. Efforts in some species have totally failed. The resulting cloned animals have nuclear DNA from the endangered species, but mtDNA from the domestic egg donor. Cloning may contribute to the future conservation of endangered animals, especially of amphibians and other non-mammalian vertebrates (Holt *et al.* [2004](#)). For example, tissues and cell lines are being collected from endangered animals and frozen in the hope that they can be used later to augment genetic diversity or to expand numbers. If cloning success improves, a captive population could be founded without removing the wild population and could be used to generate the target population size with essentially no loss of genetic diversity.

## **Jurassic Park: resurrecting extinct species from DNA**

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With current and foreseeable methods it is not feasible to resurrect extinct species from DNA. It is extremely dangerous to rely on the possible success of such methodology for conservation purposes

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A proposal by Michael Archer to resurrect the Tasmanian tiger (thylacine) from DNA has generated much news coverage and considerable controversy (Holt *et al.* [2004](#)). Russian and Japanese scientists are working on a related proposal to clone the extinct mammoth by inserting DNA extracted from mammoth remains preserved in the permafrost into elephant eggs (Smith [2006](#)). The thylacine is a large marsupial carnivore from Tasmania, Australia that became extinct in 1932. DNA is still available from bones and ethanol-preserved pups. The concept was to extract DNA, amplify it and ‘stitch’ it together to make functional chromosomes. Given that preserved DNA is highly fragmented, and that some fragments of the genome may be missing, this is most unlikely to be feasible. Alternatively, a related species, such as the Tasmanian devil, could be used as a template and homologous parts of the devil genome replaced with Tasmanian tiger DNA where available. Recent studies indicate that ancestral DNA sequences of some loci can be inferred from sequences of well-chosen extant species, and some have been proven to yield functional products (Holmes [2005](#); Hall [2006](#)).



Tasmanian tiger

Even if the first stage of the proposal works, a surrogate mother must be found. The resurrected DNA then has to be injected into an enucleate ovum, placed into the uterus of a surrogate mother and allowed to develop. All of these steps have low probabilities of success and the entire enterprise will be

very expensive. To date, some poor-quality thylacine DNA has been obtained that represents only a very small proportion of the whole genome (D. Colgan pers. comm.) and The Australian Museum, where this work began, has terminated work on the project. It would be extremely dangerous to base conservation decisions upon the assumption that such a possibility will exist in the foreseeable future. It is also dangerous to create an illusion that we can allow species to become extinct, assuming that technology can resurrect them.

## Managing inherited diseases in endangered species

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Populations of endangered species, initiated with few founders, are likely to exhibit genetic disorders at relatively high frequencies

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Many endangered populations that have originated from few founders exhibit inherited diseases, including undescended testes in Florida panthers and maned wolves, dwarfism in California condors, hernias in golden lion tamarins, hairless offspring in red ruffed lemurs, malabsorption of vitamin E in Przewalski's horse, albinism in brown bears, racoons and tigers, and missing testicles (aplasia) in koalas (Ryder [1988](#); Roelke *et al.* [1993](#); Laikre [1999](#); Seymour *et al.* [2001](#)). In each case, a deleterious allele has drifted to relatively high frequencies in a small population.

The management options are to (a) eliminate the deleterious condition, (b) minimize its phenotypic frequency or (c) ignore it. It may appear initially that inherited defects should be eliminated from populations of threatened species. However, this comes at the cost of individuals being eliminated from the breeding population, leading to loss of genetic diversity and increased

rates of inbreeding. Consequently, detailed cost–benefit analyses must be undertaken.

If the defect is dominant, then it is possible to eliminate it in one generation by simply removing affected individuals. However, most inherited defects are recessive, and most copies of the defective allele are in heterozygotes. Consequently, elimination of suspected carriers, based on pedigrees, is likely to eliminate an unacceptably large proportion of the endangered population, along with their genetic diversity. This was the case for chondrodystrophy in California condors ([Box 19.2](#)). The recommendation here was to reduce the frequency of affected individuals by avoiding matings between potential carriers, and by re-pairing individuals that had produced an affected offspring. However, if a molecular method is devised to detect carriers, the genetic costs of removing the defect could be minimized.

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### **Box 19.2 Managing a genetic disease in the California condor (modified after Ralls et al. [2000](#); Ralls & Ballou [2004](#))**

We estimated that the recessive chondrodystrophy allele had a frequency of 0.17 by assuming Hardy–Weinberg equilibrium (Example 4.5). Ralls *et al.* ([2000](#)) obtained an estimate of 0.09 using a gene drop analysis (that only accounted for alleles in known carriers and their relatives). The true estimate will lie between 0.17 and 0.09, but the difference does not affect the management conclusions reached. For simplicity, we use the former estimate in frequency computations below.

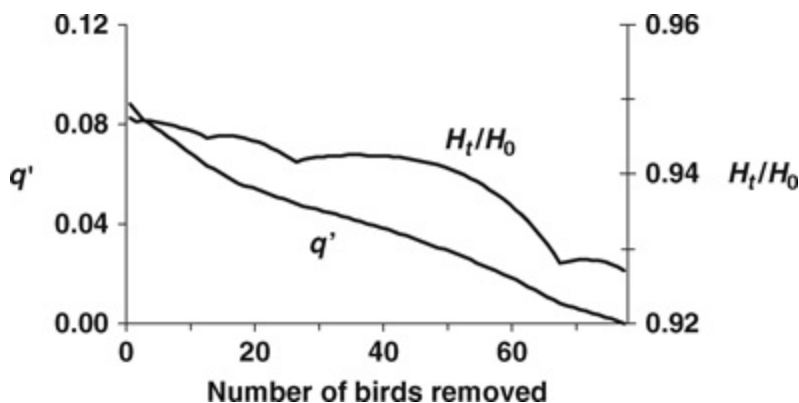


## California condor

As all affected individuals die, natural selection is expected to reduce the allele frequency from 17.2% at hatching to 14.7% in survivors (Example 6.1). With random mating, the expected frequency of affected individuals in the next generation is  $q_1^2 = 0.147^2 = 2.2\%$ .

However, by avoiding matings between known and suspected carriers, it should be possible to further reduce the phenotypic frequency. In subsequent generations, it will eventually be impossible to avoid matings between all individuals that may be carriers. However, the frequency of affected hatchlings can still be kept low by separating pairs that have an affected offspring, and pairing them with another individual that has a lower risk of being a carrier.

A detailed examination of the pedigree indicated that it would be possible to reduce the frequency of the gene to zero in the next generation, but this would involve removing 77 of 146 condors from the current reproductive pool, a cost far too high for an endangered species. Consequently, it was considered unwise to eliminate the chondrodystrophy allele. The figure below illustrates the impacts of removing suspected carriers on allele frequency and genetic diversity (after Ralls *et al.* 2000).



The pair that produced the four affected individuals was separated, and each paired with another individual presumed to be free of the *dw* allele. Pairings are now being made to minimize the risk of producing affected individuals, while at the same time maximizing retention of genetic diversity by minimizing mean kinship.



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## Summary

1. Many species have to be captive bred to save them from extinction.
2. The genetic goal of such programs is to minimize deleterious genetic changes that might occur in captivity.
3. Captive populations deteriorate genetically due to inbreeding depression, loss of genetic diversity, accumulation of deleterious mutations and genetic adaptation to captivity (leading to reduced fitness upon reintroduction to the wild environment).
4. Captive populations should be initiated with a minimum of 20–30 contributing founders to establish an adequate genetic base for the population.
5. Minimizing mean kinship is recommended to maximize retention of genetic diversity for endangered species in captivity.
6. Breeding schemes based upon maximum avoidance of group inbreeding are recommended for group breeding species where pedigrees are unknown.
7. Technologies such as artificial insemination, cryopreservation and cloning have the potential to enhance genetic management of captive populations in the future, but they have limited current use.
8. By chance, rare deleterious alleles can reach relatively high frequencies in populations derived from few founders. Detailed cost–benefit analyses are required to determine appropriate management for such conditions.

## Further reading

Ballou *et al.* (1995) *Population Management for Survival and Recovery*. Advanced treatment of topics relating to captive breeding and reintroduction; see especially the chapters by Ballou & Lacy and Princée.

Ballou *et al.* (2009) Review on genetic and demographic management of captive populations.

Botting (1999) *Gerald Durrell*. An entertaining and informative biography of the person who promoted the roles of zoos in both *ex situ* and *in situ* conservation.

Center for Plant Conservation, USA. [www.centerforplantconservation.org](http://www.centerforplantconservation.org)

Conservation Breeding Specialist Group, IUCN. [www.cbsg.org/](http://www.cbsg.org/)

Holt *et al.* (2004) Review on the potential role of cloning and assisted reproductive technologies in conservation.

ISIS Studbook CD. Contains studbooks data for many threatened species. Available through ISIS member institutions.

Kleiman *et al.* (2009) *Wild Mammals in Captivity*. Reviews on most aspects of managing captive populations of mammals in zoos.

WAZA (2005) *Building a Future for Wildlife: The World Zoo and Aquarium Conservation Strategy*. [www.waza.org](http://www.waza.org)

## Software

MateRX: Software designed to aid in choosing which pairs to breed (Ballou *et al.* 2001). [www.vortex9.org/materx.html](http://www.vortex9.org/materx.html)

PMx: Genetic and demographic management software (Pollak *et al.* 2009). [www.vortex9.org/pm2000.html](http://www.vortex9.org/pm2000.html)

SPARKS: Studbook management software (Scobie 1997).

VORTEX: Population viability analysis software that allows modelling of genetic management of captive populations (Lacy *et al.* 2005). [www.vortex9.org/vortex.html](http://www.vortex9.org/vortex.html)

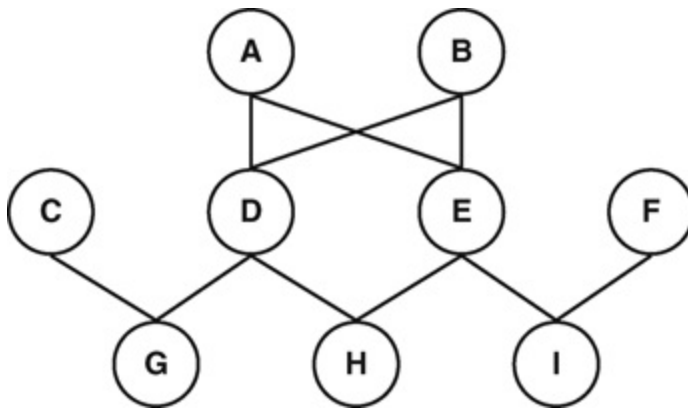
Zoological Information Management System (ZIMS): New generation of animal records keeping software that is under development.

ZooRisk: Used to evaluate the degree of endangerment of captive populations from demographic, genetic and management processes (Earnhardt *et al.* 2005).  
[www.lpzoo.com/conservation/what\\_we\\_do/projects/ZooRisk/zoorisk.html](http://www.lpzoo.com/conservation/what_we_do/projects/ZooRisk/zoorisk.html)

## Problems

**19.1** Captive breeding. By what means do captive populations deteriorate genetically?

**19.2** Kinship. What is the kinship coefficient between individuals G and H in the following pedigree?



**19.3** Kinship. Compute the kinship between Thelma and Rita in Example 19.1.

**19.4** Kinship. Compute the kinship between Thelma and herself in Example 19.1.

**19.5** Kinship. Compute the mean kinship for Rita in Example 19.1.

**19.6** Group breeding schemes. In a group breeding scheme with four populations, show the recommended mating in the first two generations and the genetic composition of the resulting populations.

**19.7** Cloning. What is the role of cloning in plant conservation? What is the role of cloning in animal conservation now? What role might cloning assume in animal conservation 20 years hence?

**19.8** Management of genetic diseases. All 125 red ruffed lemurs in captivity trace their ancestry to seven individuals, three of whom contribute more than 70% of the genes in the current population.

Some inbred families segregate for a hairless condition due to an autosomal recessive (Ryder [1988](#)). How would you genetically manage this condition? Assume that the recessive allele occurs at a frequency of about 10% in the captive population.

## Chapter 20 Genetic management for reintroduction

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Captive populations provide a source of individuals for reintroductions. Reintroductions should establish self-sustaining populations with ample genetic diversity and high reproductive fitness in the wild environment

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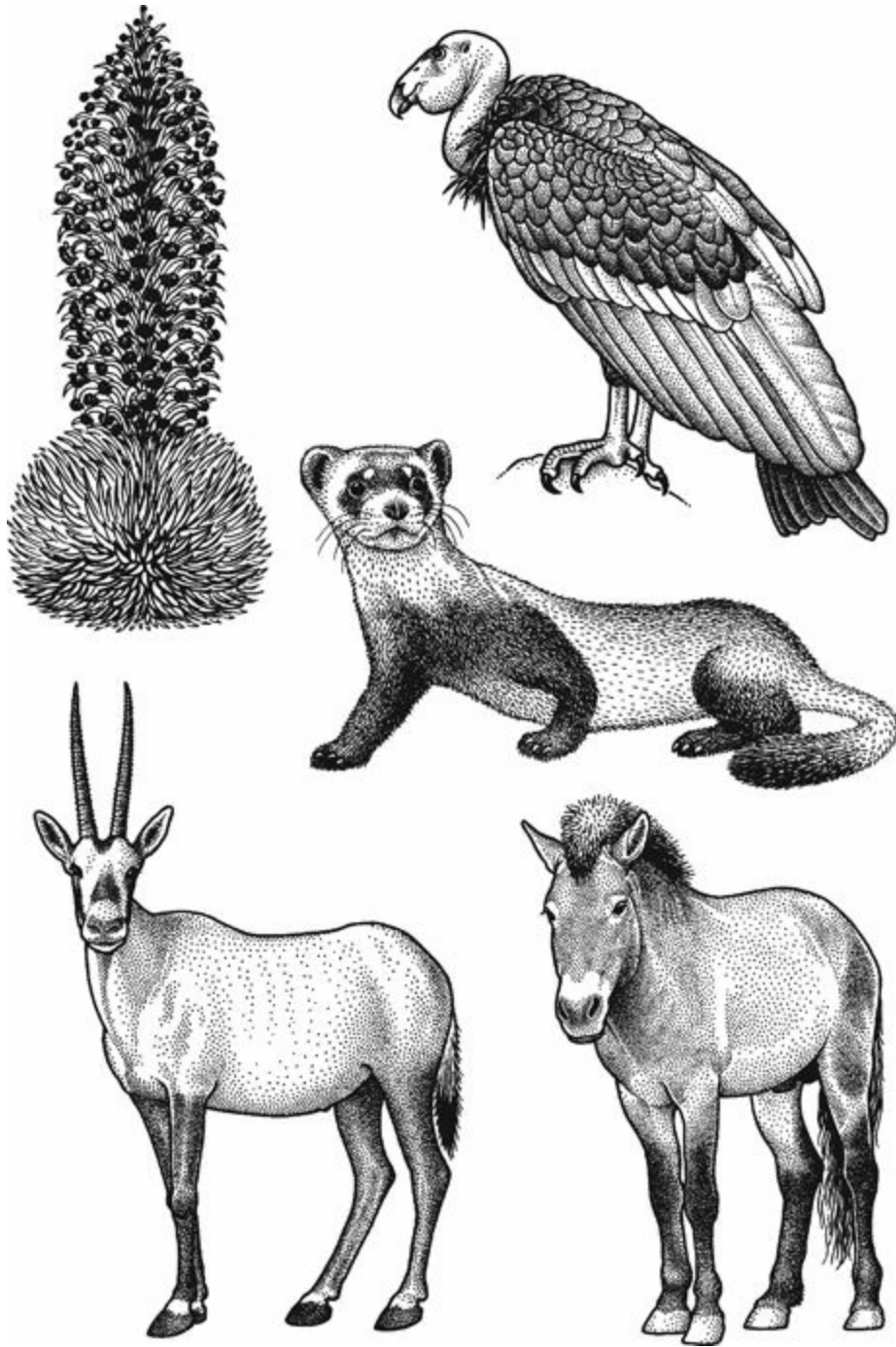
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### **Terms**

Reintroduction, supportive breeding

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A selection of endangered species that have been captive bred or propagated and reintroduced into the wild: Mauna Kea silversword (Hawaii), California condor, black-footed ferret (North America),

Przewalski's horse (Mongolia) and Arabian oryx

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## Reintroductions

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Many captive populations of threatened species are managed to preserve the option of eventual reintroduction to the wild

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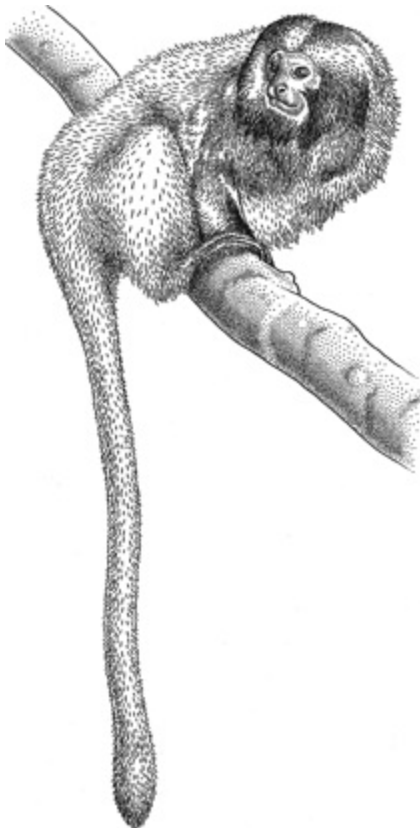
An important role of captive populations in some conservation programs is supply of animals to re-establish, or supplement existing wild populations. While some reintroductions are carried out after only brief periods in captivity (e.g. black-footed ferrets, California condor, Lord Howe Island woodhen), many may occur after long captive histories. Conditions for reintroduction are currently not favourable for most species, as wild habitat has been destroyed, or the original threatening process still persists (Kleiman *et al.* 2009). For many, a decline in the human population will be required followed by release of wild habitat before they can be reintroduced ~100–200 years in the future (Soulé *et al.* 1986). Nevertheless, many captive breeding programs aim to retain sufficient levels of genetic diversity and demographic viability to retain the option.

The golden lion tamarin represents, arguably, the most extensively managed captive breeding and reintroduction program and illustrates many of the issues involved (Box 20.1).

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**Box 20.1 Genetic management of captive, reintroduced and wild populations of golden lion tamarins (Ballou & Lacy 1995; Grativol et al. 2001)**

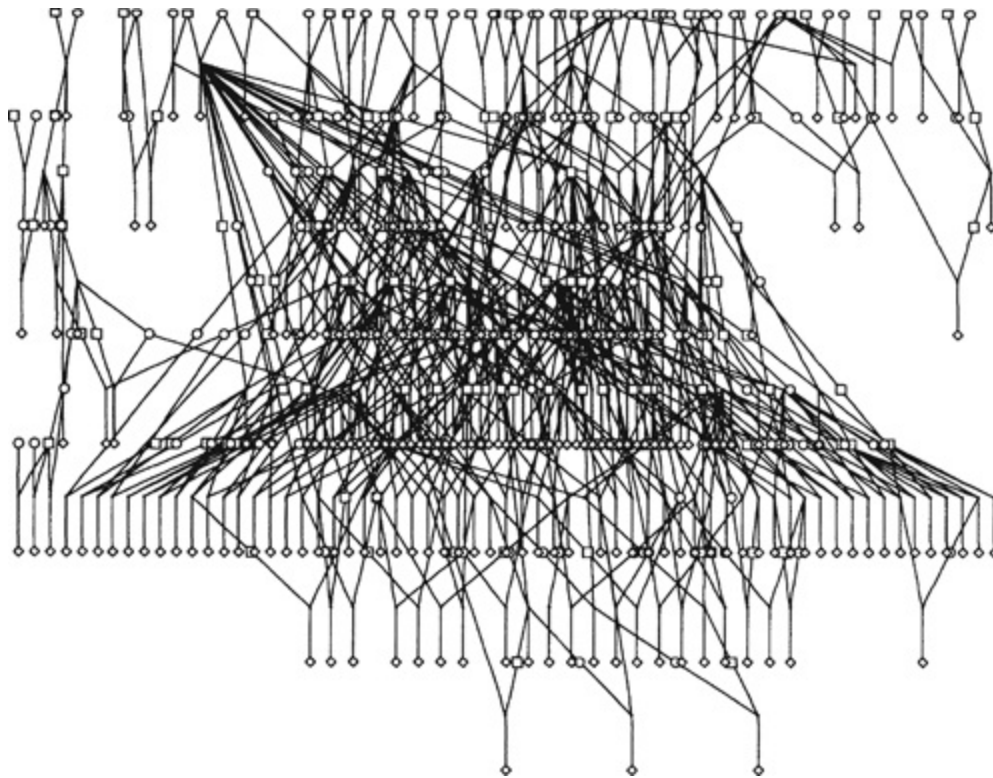
Golden lion tamarins are small, arboreal, monogamous primates from Brazil. Tamarin numbers have declined and the species has become endangered since their habitat in the Atlantic Rainforest has been fragmented and reduced to less than 2% of its original area. The Golden Lion Tamarin Conservation Program is a collaboration including the Smithsonian National Zoological Park, the Golden Lion Tamarin Conservation Association and the Brazilian Government. It is the largest global captive breeding and reintroduction program and integrates captive breeding, reintroduction, translocation, studies on the ecology of wild tamarins, habitat restoration and community conservation education (Kleiman & Rylands 2002).



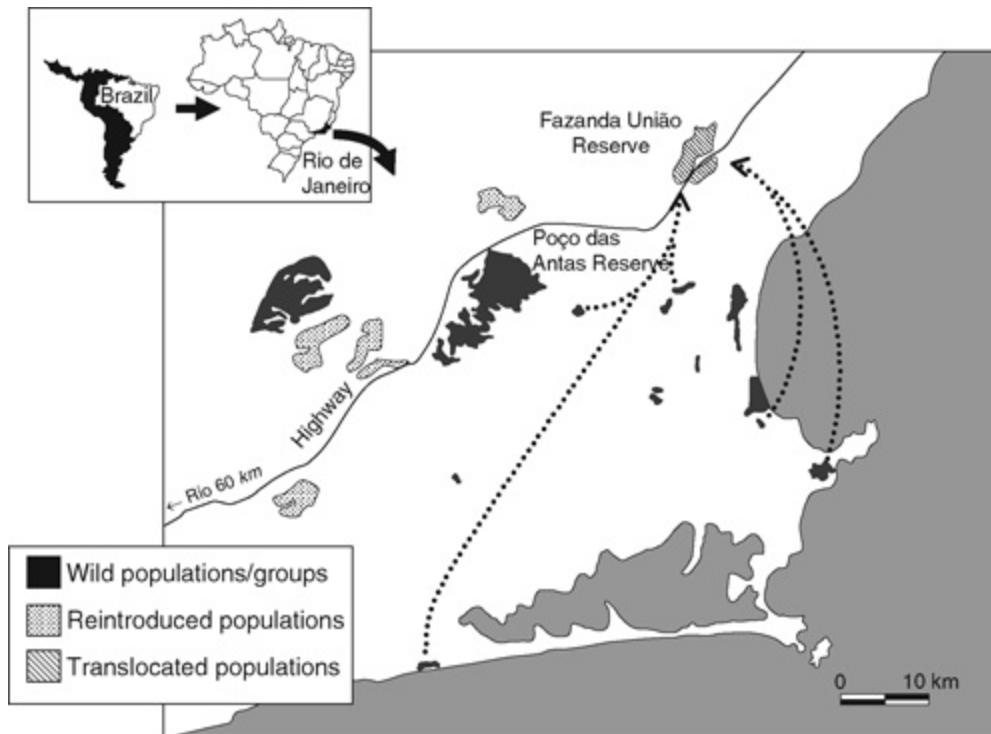
The captive population consists of about 500 individuals located in 154 zoos worldwide. The concept of genetic management by mean kinship



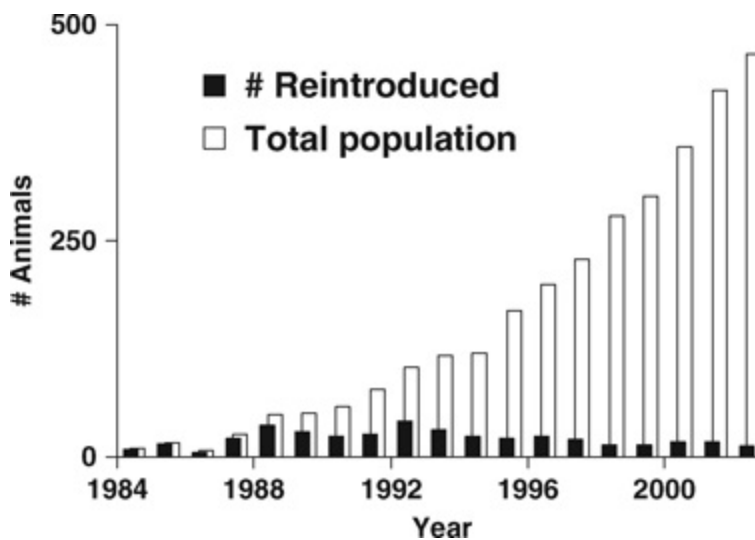
was originally developed for this complex population. Although the population is descended from 48 founders, about two-thirds of the genes derived from just one prolific breeding pair, prior to genetic management (see pedigree below). However, due to subsequent careful genetic management, the level of inbreeding in the population is now only 2.6%.



The wild population consists of about ~1600 individuals dispersed among 18 isolated populations (see map), the largest being Poço das Antas Biological Reserve containing ~390 tamarins. Translocation of animals from the most severely threatened populations to a newly established reserve established a second large protected population. Molecular analyses indicate that small populations have lost genetic diversity and become genetically differentiated from each other. This information will be used to design a program of regular translocations among fragments to minimize inbreeding and maximize the effective size of the entire population, as inbreeding reduces juvenile survival in this species (Fig. 13.1). Further, 21 habitat corridors have been planted to connect fragments (A. M. de Godoy Teixeira pers. comm.).



The reintroduction program was initiated in 1983 to re-establish populations in areas of their former range. A total of 153 tamarins have been released, and the reintroduced population has flourished, reaching between 530 and 750 at the end of 2005 with a growth rate of 25% per annum. Numbers reintroduced in the figure below refers to those surviving to the end of the year of reintroduction.



The reintroduced population is genetically monitored in a similar

manner to a zoo population. Until recently, weekly monitoring tracked parentage, birth and death dates, and migration events among groups. These are recorded in a studbook database and used to identify relationships within the population. Based on pedigrees of both the reintroduced and captive populations, release of animals that are (a) genetically valuable to the captive population or (b) closely related to previously reintroduced animals is avoided. Additional reintroductions have been put on hold as the reintroduced populations have grown to capacity.

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In the previous chapter, we discussed issues in the genetic management of captive species from foundation to maintenance. Here we focus on ways to limit genetic change during captivity, so that the long-term viability of reintroduced populations is maximized. Further, we consider practical questions in reintroduction; which and how many individuals should be released, where and how many wild populations should be re-established, and what management should be applied to the new populations.

The option of release is, regrettably, being de-emphasized in some programs. This may be considered a ‘policy of despair’. We argue that reintroduction must remain the ultimate goal for the vast majority of captive-bred species. However, the success of reintroductions is jeopardized by genetic deterioration in captivity, and management programs must be implemented to mitigate this loss of genetic ‘health’.

## **Genetic changes in captivity that affect reintroduction success**

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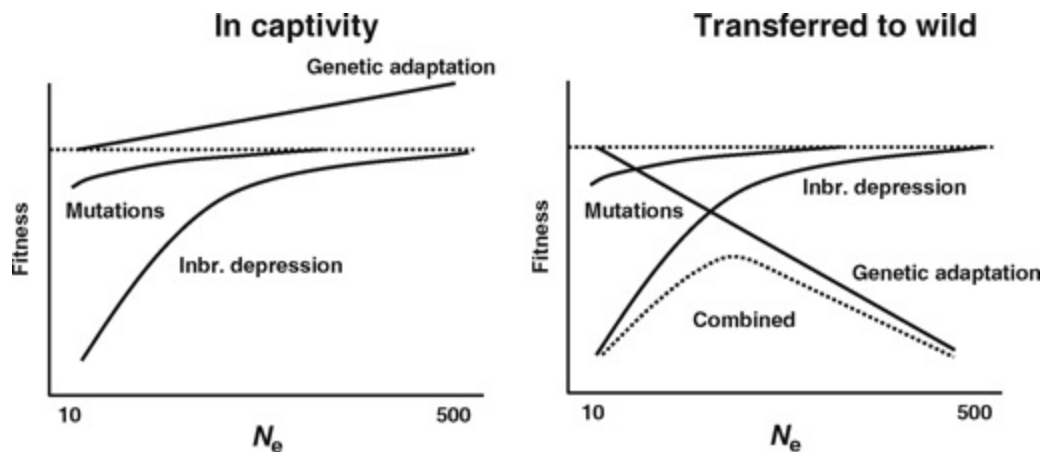
Inbreeding depression, loss of genetic diversity, genetic adaptation to

captivity, relaxation of natural selection and accumulation of new deleterious mutations reduce reintroduction success

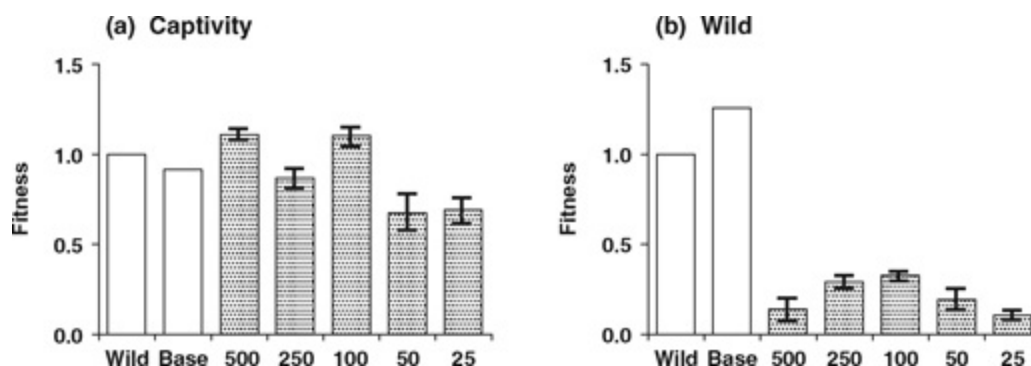
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So far, we have considered genetic deterioration during the decline of the original natural population, and during founding, growth and maintenance of the captive colonies. In this chapter, we consider the impact of genetic adaptation to captivity and means for minimizing it. Translocation from the wild to captivity represents a dramatic environmental change, altering the selective pressures acting upon a population. Populations will evolve to adapt to their new environments, but such adaptation is overwhelmingly deleterious when populations are returned to the wild (see below).

The predicted relationships of reproductive fitness with effective population size ( $N_e$ ) due to the impacts of inbreeding depression, accumulation of deleterious mutations and genetic adaptation to captivity are shown in [Fig. 20.1](#) for captive populations and for reintroduced populations. The effects of inbreeding depression, loss of genetic diversity and mutational accumulation are all more severe in smaller than in larger populations and in harsher, wild environments than in captivity. Conversely, genetic adaptation to captivity is greater in larger than in smaller populations ([Chapters 8 and 11](#)). While this is beneficial to populations in captivity, its deleterious effects may be severe when populations are returned to the wild. Experimental results in [Fig. 20.2](#) confirm the basic predictions in [Fig. 20.1](#).



**Fig. 20.1** Genetic deterioration in captivity and its impact on reintroduction success: predicted relationships of reproductive fitness with effective population size ( $N_e$ ) due to the impacts of inbreeding depression, accumulation of deleterious mutations and genetic adaptation to captivity. Reproductive fitness under captive conditions is shown on the left, and for captive populations transferred to the wild on the right. The straight dotted lines are the fitness of a large wild population. The *combined* curve is the cumulative impact of inbreeding depression and genetic adaptation to captivity. A long-term time frame is being considered (~50 generations).



**Fig. 20.2** Genetic deterioration in captivity and its effects on reproductive fitness in populations translocated to the 'wild' (Woodworth *et al.* 2002). Reproductive fitness at generation 50 of fruit fly populations maintained in benign captive conditions with effective population sizes of 25, 50, 100, 250 and 500. Fitness under captive conditions was reduced by inbreeding depression, but increased by genetic adaptations to captivity, resulting in lowest fitness in the smaller populations. Fitness under crowded, competitive 'wild' conditions was markedly reduced in all treatments due to inbreeding depression and to genetic adaptations to captive environmental conditions

that were deleterious in the 'wild'. No contributions from accumulation of new deleterious mutations were found in these populations (Gilligan *et al.* [1997](#)).

## Genetic adaptation to captivity

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Genetic adaptations to captivity are overwhelmingly deleterious when populations are returned to the wild

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Evolutionary adaptation in captivity has been recognized since the time of Darwin. Until recently it has been considered only a minor problem in captive breeding. There is now evidence showing that it can be a major threat to the success of reintroductions (Frankham [2008](#)).

When wild populations are brought into captivity, the forces of natural selection change. Populations are naturally or inadvertently selected for their ability to court, mate, breed and develop in the captive environment. Flightiness in animals such as antelopes, gazelles, kangaroos and wallabies is naturally selected against when flighty animals die after running into fences, etc. Tamelessness is actively favoured by some keepers. Prey capture and predator avoidance are no longer positively selected, while veterinary care and hygiene remove most selection for disease and parasite resistance.

Adaptation to captivity has been detected in all outbreeding species where it has been studied, including mammals, fish, insects and plants (see Frankham [2008](#)). The extent of change may be very large. Large white butterflies exhibited a 13-fold increase in fecundity over 100–150 generations in captivity, wild rats a three-fold increase in fecundity over 25 generations

and barley a doubling of fitness over 60 generations.

This adaptation is overwhelmingly deleterious when captive populations are returned to the wild, as documented in turkeys, amphibians, plants and many species of fish and biocontrol insects (see Frankham 2008). For example, lifetime reproductive success of hatchery fish stocks returned to the wild declined exponentially by 38% per generation, compared to wild adults (Araki *et al.* 2007). Further, Myers & Sabath (1980) reported that the success of biological control programs was negatively related to time in captivity.

Genetic adaptation to captivity appears to be due predominantly to alleles that were previously rare and deleterious in the wild, but become favoured in captivity (Frankham 2008). For example, a rare  $\alpha$ -glycerophosphate dehydrogenase allele with lowered enzyme activity in screwworm flies increased markedly in frequency in captivity but reduced wild fitness as a consequence of compromised flying ability (Bush *et al.* 1976).

## Minimizing genetic adaptation to captivity

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Genetic adaptation to captivity can be reduced by limiting the number of generations in captivity, the magnitude of selection and the size of captive populations

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Given the adverse effects of genetic adaptation to captivity on reintroduction success, it is important to devise means to minimize its impacts. After  $t$  generations, genetic adaptation to captivity ( $GA_t$ ) in a population of size  $N_e$  is predicted to be (Equation 15.5):

$$GA_t \sim Sh^2 \sum \left(1 - \frac{1}{2N_e}\right)^{t-1}$$

where  $S$  is the selection differential and  $h^2$  the heritability.

Consequently, adaptation can be reduced by minimizing:

- number of generations in captivity ( $t$ )
- the magnitude of selection in captivity ( $S$ )
- genetic variation ( $h^2$ ) within populations
- the size of captive populations ( $N_e$ ).

If immigration from the wild is feasible (it rarely is), genetic adaptation can also be minimized by:

- maximizing the proportion of wild immigrants, especially in recent generations.

The most effective means for minimizing genetic adaptation is to restrict the number of captive generations. This is widely practiced in plants, by storing dormant seeds. As of early 2006, the Millennium Seed Bank project held seeds of over 14 000 species in cold storage ([www.kew.org/msbp/index.htm](http://www.kew.org/msbp/index.htm)). Seed banking also has a major role in the program of the Center for Plant Conservation in the USA ([www.centerforplantconservation.org](http://www.centerforplantconservation.org)).

Minimizing generations in captivity can also be achieved by cryopreservation (Johnston & Lacy 1995). This is a feasible option for plants where the technology appears to transfer well across species (Touchell & Dixon 1993). However, cryopreservation only works for a small minority of animal species, mostly those closely related to domestic species (Chapter 19). Breeding from older animals can be used to extend the generation interval, but is generally impractical, as animals that do not breed when young often breed poorly when older (Chapter 19).



Low numbers of generations in captivity have been utilized for Lord Howe Island woodhens, black-footed ferrets and California condors. Conversely, Père David's deer has been in captivity for hundreds of years and Przewalski's horse for most of the twentieth century.

Selection can be minimized by making the captive environment similar to the wild environment. However, duplicating a harsh natural environment is unrealistic in captive breeding. The first priority is to establish secure and viable populations of the threatened species by removing predators, minimizing parasites and disease and generally providing a benign environment.

Genetic adaptation can also be reduced by managing the population to ensure equal representation of founder alleles and avoiding preferential breeding of individuals. Genetic management using mean kinship can halve the intensity of selection. This has been verified in fruit flies, but it did not translate into significant benefits in wild fitness (Frankham *et al.* [2000](#); Frankham [2008](#)).

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Minimizing kinship can halve genetic adaptation to captivity

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## **Population fragmentation as a means for minimizing genetic adaptation to captivity**

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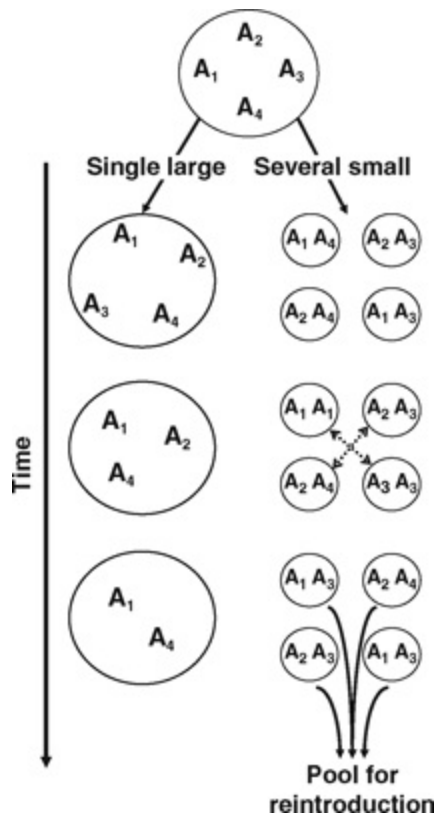
Fragmentation of populations, with occasional exchanges of individuals,

reduces genetic adaptation to captivity and retains more overall genetic diversity than in a single population of the same total size

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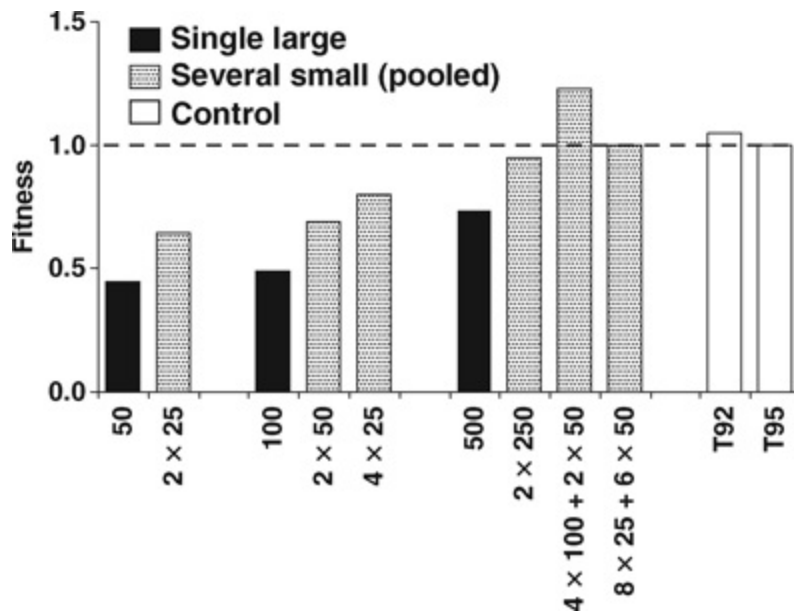
What is the best captive management to use for species that will be reintroduced? Neither large, nor small populations are ideal to simultaneously maintain genetic diversity, avoid inbreeding depression and avoid genetic adaptation to captivity (see [Fig. 20.1](#)).

A compromise may be achieved by maintaining a large overall population, but fragmenting it into partially isolated sub-populations ([Fig. 20.3](#)). Genetic adaptation is reduced by the fragmentation, but it results in increased inbreeding. The sub-populations are initially maintained separately until inbreeding builds to a level where it is of concern. This may occur as  $F$  approaches 0.2, although populations should be monitored for signs of fitness decline prior to this. Immigrants are then exchanged among sub-populations. The sub-populations are again maintained in isolation until inbreeding accumulates. This structure is expected to maintain more genetic diversity than a single population of the same total size and to exhibit less deleterious genetic adaptation to captivity. There will be low but tolerable levels of inbreeding depression.



**Fig. 20.3** Single large or several small: alternative genetic management options for captive populations. *Several partially isolated, fragmented sub-populations, when pooled, should maintain more genetic variation, show less genetic adaptation to captivity and have lower inbreeding coefficients than a single large population with the same total size.*

When all sub-populations are combined (e.g. to produce animals for reintroduction) the pooled population has a lower level of inbreeding and more genetic diversity than would a single large population of the same total size. These predictions have been validated in experiments with fruit flies (Fig. 20.4). A critical requirement in the use of fragmentation is that none of the sub-populations dies out. Thus, it can be used in captivity where probabilities of population extinction can be kept low, but is not applicable for wild populations where extinction probabilities are high.



**Fig. 20.4** Experimental evaluation of population fragmentation for the captive management of populations destined for reintroduction. Reproductive fitness under ‘wild’ conditions for fruit fly populations previously maintained for 50 generations under benign captive conditions is shown for single large populations, compared to several small pooled populations of equivalent total size. The several small populations were pooled after generation 50 (Morgan *et al.* 1998). *In all cases the fitness was greater in the pooled populations than in the single large populations.*

This structure also satisfies other requirements in captive breeding. A fragmented structure involves less frequent migration, and reduces costs and the risk of injury and disease transmission.

How would a fragmented structure be implemented? Initially it is envisaged that movement of animals among institutions for genetic management would cease, but that populations within institutions would be managed by minimizing mean kinship. When inbreeding levels reach ~0.2, nearby zoos would exchange animals, for example, all breeding males. The new outbred populations would again be maintained in isolation until inbreeding again rises to ~0.2. Exchange of animals would then be among zoos that are more distant. The process would be similar to maximum avoidance of inbreeding with groups (Box 19.1). The number of generations

between animal transfers depends on the  $N_e$  of each population, and will be more frequent for smaller than for larger populations.

This process already appears to be happening on a global scale due to the regionalization of many captive breeding programs. Most animal transfers take place within regions and exchanges between regions are pursued only when genetic diversity becomes low.

While this strategy is not officially sanctioned, it has both conceptual and practical merit.

## **Recovery from genetic adaptation to captivity in reintroduced populations**

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Natural selection in the wild will operate to reverse deleterious adaptations that occurred in captivity, provided that there is genetic diversity and the population has sufficient reproductive fitness to persist in the wild

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After being ‘reintroduced’ fruit fly populations that had been kept in captivity with  $N_e = 500$  for 50 generations had only 14% of the fitness of a wild population. However, they displayed a five-fold improvement over 12 subsequent generations to reach a fitness 70% that of the wild population (Woodworth *et al.* [2002](#)). Natural selection will operate in a similar manner in all reintroduced populations, provided they persist and have genetic diversity.

## **Genetic management of reintroductions**

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The genetic objective in reintroduction programs is to re-establish populations with high genetic diversity, using individuals predicted to have maximal reproductive fitness under wild conditions

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There are many practical considerations when a reintroduction is contemplated. Genetics may play a relatively minor role in some decisions (Kleiman *et al.* [2009](#)), but should not be ignored, as often occurs. There will be a higher probability of success if all issues are considered (see below). Genetics can contribute to:

- choice of sites for reintroduction
- choice of genotypes to be released
- choice of individuals, and numbers to release
- decisions on the number of release sites
- genetic management of released populations.

These issues are considered in turn below.

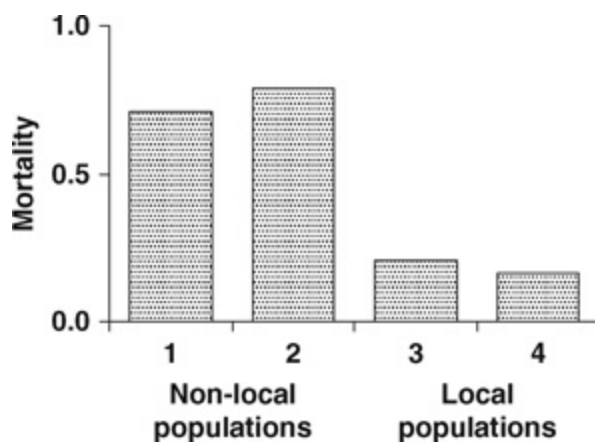
## **Choosing sites for reintroduction**

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Reintroductions and introductions should be carried out in sites where the species has the best chance of surviving in the wild

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The reintroduction site should match, as closely as possible, the environment to which the population had adapted, prior to captive breeding. The site should be within the previous range of the species and ideally be prime, rather than marginal, habitat. This will minimize the extent of adaptive evolution required in the reintroduced population. For example, in butterfly reintroductions the site should support the food-plants to which the species is best adapted. Plants should be genetically adapted to the soil, day length and climatic conditions of their reintroduction site. For example, mortality in the plant *Nasella pulchra* was greater in non-local than local populations (Fig. 20.5). Similar results have been found in many other plant studies (Guerrant 1996; Montalvo & Ellstrand 2000). Further, reintroduced white storks native to northeastern Europe had much higher fitness in Sweden than did North African birds (Olsson 2007).



**Fig. 20.5** Populations adapted to local conditions have higher fitness than those from distant sites. Mortality in four *Nasella pulchra* plant populations planted at Winters, California (Knapp & Dyer 1998).

## Choosing genotypes and individuals to reintroduce

Reintroduction programs should use healthy individuals with as much reproductive ability and genetic variation as possible

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All other factors being equal, healthy individuals with high reproductive potential, low inbreeding coefficients and extensive genetic diversity should be chosen for reintroduction. Inbred deer mice, snails, American kestrels and plants were found to have lower survival than their outbred counterparts when released into the wild (Frankham [1995a](#); Kephart [2004](#); Vilas *et al.* [2006](#)). Populations of rose clover with higher genetic variation had greater success when translocated into new environments than populations with less genetic variation (Martins & Jain [1979](#)).

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When choosing animals for reintroduction, the genetic impacts of both adding them to the new population, and of removing them from the captive population, must be evaluated

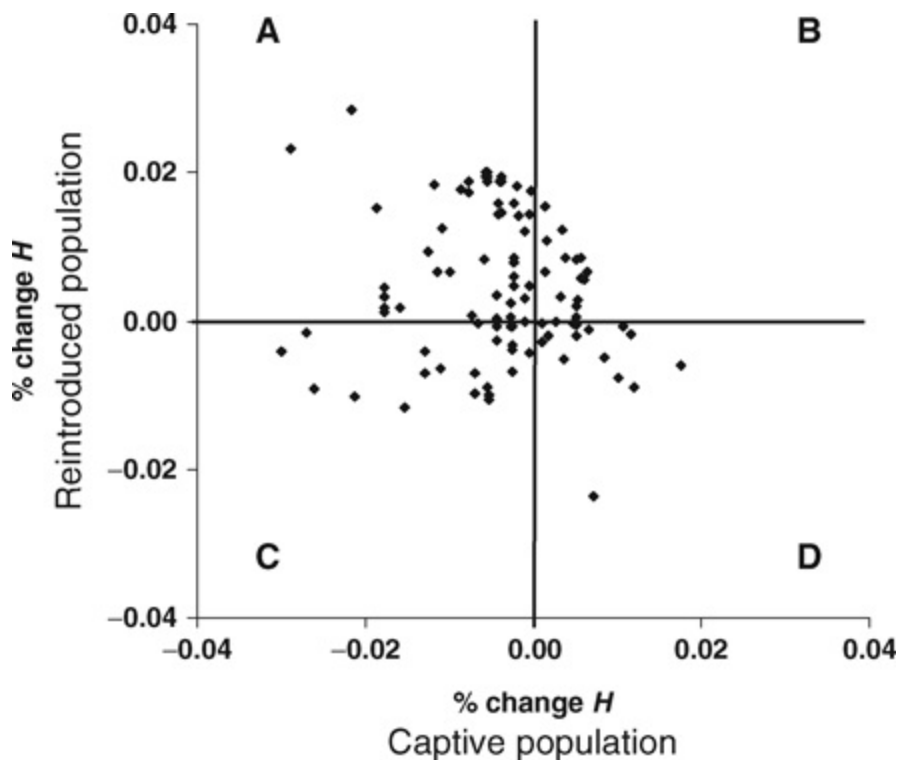
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When an individual is reintroduced, its genetic diversity is added to the wild population, but removed from the captive population. Thus, care must be taken to evaluate the impact of reintroducing individuals on both the captive and the wild population (Earnhardt [1999](#)). For example, the release of genetically valuable animals will have detrimental effects on the captive population and their value may be lost altogether if reintroduction mortality is high. Conversely, an otherwise ideal reintroduction candidate may be closely related to animals previously released and its introduction may actually increase the level of inbreeding in the wild population.

The interests of the two populations frequently conflict, as illustrated for California condors in [Fig. 20.6](#). Individuals (points) in quadrant A are those whose reintroduction would benefit the genetic diversity of the reintroduced



population, but be harmful to the captive population. These are genetically valuable captive animals with few relatives in the wild population. Individuals in quadrant B will benefit both the wild and the captive population. These are genetically over-represented captive animals with few reintroduced relatives. Release of individuals in quadrant C would be detrimental to both populations, as they are valuable in the captive population but have many reintroduced relatives. Individuals from quadrant D are over-represented in both populations. Their release is beneficial to the captive population, but detrimental to the reintroduced population.



**Fig. 20.6** Changes in genetic diversity in the captive and re-established populations resulting from releasing individual California condors. Each point shows how reintroducing that individual will affect the heterozygosity ( $H$ ) of the re-established population and how its removal will affect the heterozygosity of the captive population.

Because of the high mortality risks commonly associated with release of captive-bred animals, individuals of type D should be used initially. This has been done with golden lion tamarins, California condors, black-footed ferrets and Przewalski's horses. Survival and reproduction of individuals in the

reintroduced population must be carefully monitored. When these have improved, more valuable animals may be added until the full range of genetic diversity in the captive population is represented in the wild. Without careful pedigree or genetic marker monitoring, genetic composition of reintroduced populations may be distorted, as occurred in both Mauna Kea silverswords and Galápagos tortoises (Milinkovitch *et al.* [2004](#)).

## **Choice of individuals to reintroduce when several captive populations are available**

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When several captive populations exist, individuals for reintroduction may come from a single population, from multiple populations, or from crosses among individuals from different populations

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To determine the best choice among the above options for reintroductions requires an evaluation of their probabilities of success (Guerrant [1996](#)). Where there is no relevant *a priori* information, trials are advisable to compare the fitness in the reintroduction site of the candidate populations and their crosses.

For species with strong local adaptation, the single best adapted population should be used for reintroduction. This requires accurate records of the sources of populations, and presumes that the environment has not changed substantially. The latter presumption is often untrue, as the initial endangerment of species frequently arises from environmental deterioration.

If the environment has changed, reintroduction success should be highest when using individuals from crosses among populations. Further, crosses among different long-term captive populations should have improved wild

fitness due to the masking of deleterious, partially recessive alleles that contribute to genetic adaptation to captivity (Woodworth *et al.* [2002](#); Frankham [2008](#)).

If the species is a natural outbreeder and the captive populations are inbred, then use of crosses between populations should maximize reintroduction success. Reintroduction of individuals from several small differentiated populations, each with low genetic diversity, has been used for collared lizards in Missouri, USA (Templeton [1986](#)). However, care must be taken to avoid outbreeding depression in such cases. The most prudent approach is to proceed with reintroductions on an experimental basis, monitoring survival and reproductive success of pure and hybrid individuals. This was undertaken for the Florida panther, following the introduction of individuals from the Texas sub-species (Pimm *et al.* [2006](#)). Such data can then be used to decide upon the optimum approach for further reintroductions.

## **How many reintroduced populations should be established?**

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There are genetic and non-genetic advantages in establishing several reintroduced populations

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Establishing a number of reintroduced populations to maximize the numbers of individuals will minimize loss of genetic diversity and inbreeding if individuals are translocated among the different sites (Leberg [1990b](#)). It should also reduce the risk of extinction due to catastrophes and environmental stochasticity. For example, at least 10 reintroduced populations of the black-footed ferret have been recommended ([Chapter 17](#)).

## **Genetic management of reintroduced populations**

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Once populations are reintroduced and self-sustaining, they should receive the full range of available genetic diversity

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As the re-established population becomes self-sustaining, then the more valuable animals of type B, and later of type A, should be released ([Fig. 20.6](#)). Eventually the wild population will receive all the genetic diversity available from the captive population, inbreeding will be minimized and the reproductive fitness increased. Subsequent genetic management follows that for wild populations, discussed in [Chapter 17](#).

## How successful are reintroductions?

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Reintroduction is a complex process with a relatively low success rate to date

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Reintroduction of threatened species is a complex process requiring considerable understanding of species' biology and ecology. Even introductions of widespread and successful pest species, such as rabbits and European starlings, have failed (Fenner & Ratcliffe [1965](#); Fyfe [1978](#)).



Bison

Beck *et al.* (1994) considered only 11% of reintroduction programs to be successful, based upon the criterion that reintroduced populations had reached at least 500 individuals, free of human support. Successes have included bison, Arabian oryx, Alpine ibex, golden lion tamarin, bald eagle, peregrine falcon, Aleutian goose and Galápagos tortoise. These successful projects were typified by release of larger numbers of animals and over longer periods. Successful projects frequently included more local employment and community education programs than other projects. A second study classified 26% of reintroductions plus some translocations as successful, 27% as failures and 47% as unknown at the stage of publication (Fischer & Lindenmayer 2000). The success rate was higher when the source population was wild (31%) than when it was captive (13%), when a large number of animals was released and when the cause of the original decline was removed. The success rates of recovery efforts in the wild are also low (Chapter 17).

The relatively low success rates for reintroductions are not surprising since some programs have not been allowed sufficient time to succeed. Further, some programs have been rather cursory. Animals have been reintroduced and left to fend for themselves. Reintroduction success should improve substantially as the underlying science advances.

We are not aware of any quantitative evaluations of reintroduction success in plants. However, the desirability of high genetic variation in reintroduced populations is widely accepted (Huenneke 1991; Fiedler & Karieva 1998; Helenurm 1998). Experiments with sweet vernal grass demonstrated a fitness

advantage of genetically variable (sexual) over genetically uniform (asexual) progeny, under natural field conditions (Ellstrand & Antonovics [1985](#)).

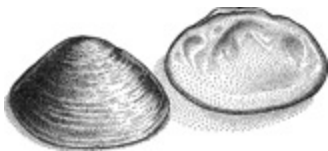
## Supportive breeding

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Some wild populations are regularly augmented using captive-bred animals (supportive breeding)

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Augmentation of non-sustaining wild populations is sometimes required. The wild population of the nene (Hawaiian goose) is not self-sustaining and is continuously augmented using captive-bred animals (Black [1995](#)). Over the past several years, over 1 million juveniles of more than a dozen species of endangered freshwater mussels have been released into rivers in the eastern United States. Captive propagation and release are critical to their conservation. Hatchery fish stocks are widely used to augment a large number of wild fish species, especially those favoured by anglers.



Cumberlandian combshell mussel

Unfortunately, many fish hatcheries have not practiced enlightened genetic management. Often the effective sizes of hatchery populations are very low or there is extreme selection for adaptation to an environment that is very different from the release environment. In such cases, supportive breeding is likely to lead to (a) reduced wild effective population sizes, (b) reduced reproductive fitness resulting from genetic adaptations to captivity that are

deleterious in the wild, and perhaps to (c) inbreeding depression (Ryman *et al.* 1995; Ford 2002). For example, Araki *et al.* (2007) reported rapid, cumulative genetic reductions in wild fitness of salmonid fish from captive breeding, when compared to wild stock.

## Case studies in captive breeding and reintroduction

### Arabian oryx

The Arabian oryx was driven to near extinction in the wild because of hunting with increasingly sophisticated weapons. The species was saved by capture of the last wild animals in 1962, followed by a captive breeding program based on 10 founders, primarily at the Phoenix Zoo, Arizona. There was a steady increase in numbers and implementation of a genetic management program. With funding and co-operation from the Sultan of Oman, a reintroduction program was successful (Stanley Price 1989). Ten animals were initially introduced into pens, followed by gradual release in their native range. Local tribesmen were employed in management and tracking of the oryx. Released animals showed good survival and reproduction, with relatively normal behaviour. By 1996, there were approximately 400 animals in the wild, ranging across the 26 636 km<sup>2</sup> Arabian oryx sanctuary.



Arabian oryx

Unfortunately, poaching recurred and the wild population was so depleted that most individuals have been returned to captivity (Gorman 1999). Other reintroduced populations persist in Saudi Arabia (populations with 800 and 129 individuals), in the United Arab Emirates (241 animals) and Oman (94 individuals, but all except four were males as poachers prefer females) (Chassot *et al.* 2005; El Alqamy 2006).

Microsatellite analyses have revealed loss of alleles due to the founder bottleneck, errors in the pedigrees and the occurrence of simultaneous inbreeding and outbreeding depression in the species (Marshall *et al.* 1999; Marshall & Spalton 2000). The population is polymorphic for a chromosomal translocation which could cause outbreeding depression, and may indicate that the founders derived from diverged sources (Benirschke & Kumamoto 1991).

## **Black-footed ferret**

The black-footed ferret was reduced in numbers through habitat loss, and especially from vigorous efforts by cattle ranchers to exterminate their prairie dog prey (Clark 1994). The last wild population was discovered in Wyoming in 1981 and a captive breeding program begun in 1985. When distemper was found to be killing animals, the last wild individuals were captured. The captive breeding program was founded from 18 individuals (including two mothers with their litters) that contributed unequally. By 2007, the population had descended from only 10 founders (Russell *et al.* 1994). The captive population has increased to about 300 adults, and has been genetically managed to minimize mean kinship.



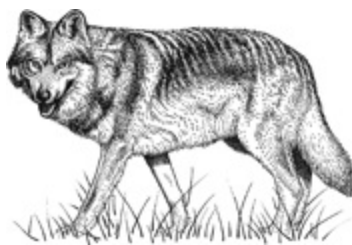
Black-footed ferret



A reintroduction program commenced in 1991, the eventual objective being to establish 10 populations with a total of at least 1500 animals. About 1 185 captive-bred ferrets were introduced into the wild in 1991–99. Currently reintroduction has established >11 populations (Wisely *et al.* 2008). In 2000, over 85 litters were born in the wild, but only two of the reintroduced populations are considered successful. Microsatellite analyses show that genetic management of the captive population was successful in retaining the levels of genetic diversity that survived the initial bottleneck. The reintroduction program has also been successful in transferring the captive population's genetic diversity into the reintroduced South Dakota populations. However, genetic diversity is low in the Wyoming population which has been small for a long period without further reintroductions (Wisely *et al.* 2008).

## **Mexican wolf**

By the 1970s, the Mexican wolf had been driven almost to extinction in the United States and Mexico. To save them from extinction, a captive breeding program was established with three wolves captured in Mexico between 1977 and 1980 (Certified or McBride lineage). Two other populations founded with individuals of uncertain ancestry were also established, but the populations were kept separate due to the questionable ancestries. Inbreeding accumulated rapidly and average  $F$  peaked at 0.18, 0.61 and 0.26 in the three lineages. Microsatellite analyses demonstrated that all were derived from pure Mexican wolves (Chapter 21). The lineages were combined, beginning in 1995, resulting in a population with an inbreeding coefficient of only ~ 0.085 (Hedrick & Frederickson 2008).



Mexican wolf

The reintroduction program began in 1998 and as of late 2006 there were 40–50 wolves in the wild in Arizona and New Mexico (Hedrick & Frederickson 2008). Inbreeding depression (fewer pups) has been documented in the wild. There are concerns about the viability of the reintroduced population due to high death and removal rates associated mainly with human conflicts and limitations on further reintroductions. The captive population continues to be managed to supply wolves for potential future reintroductions. In 2005, there were approximately 300 Mexican wolves managed in 47 facilities in the United States and Mexico.

## **Przewalski's horse**

Przewalski's horses (also known as Takhi) have been restricted to captivity for much of the twentieth century (Boyd & Houpt 1994). A captive population was founded from 12 individuals, plus two or more domestic mares (a separate species with a different chromosome number). Numbers in captivity are now approximately 1500. The captive population has an average inbreeding coefficient of  $F = 0.20$  (Box 12.1). It has been managed to minimize inbreeding, equalize founder representation and reduce the genetic inputs from the domestic mares. Between 1992 and 2004, 90 horses from 24 institutions were released in the Gobi Desert, at the site where the species was last seen in the wild (Van Dierendonck & de Vries 1996). Recommendations made in 1985 stipulated that the reintroduced horses should have genetic contributions from all the founders, but with no individual having more than 15% from the domestic species, and that the average inbreeding coefficient of potential offspring was not to exceed 0.30 (FAO 1986). As of 2006, over 200 individuals exist in the wild in three different reserves.



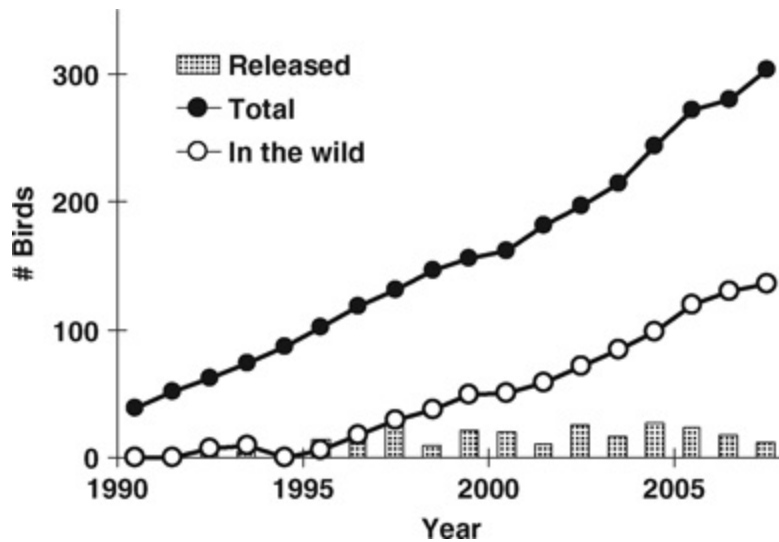
Przewalski's horses

## California condor

The California condor was reduced in population size due to habitat loss, DDT pollution and lead poisoning (from eating shot wildlife carcasses) (Ralls *et al.* 2000). After much controversy, the last wild birds were captured and a breeding and reintroduction program, based on 14 founders, was instituted at San Diego Wild Animal Park, Los Angeles Zoo and the World of Birds. Captive numbers have steadily increased and a limited number of releases have occurred (Fig. 20.7). The major initial objective was to increase the size of the population as rapidly as possible. Reintroduction to the wild initially met with limited success due to deaths from lead poisoning, collisions with electricity power lines and poisoning due to ingestion of vehicle antifreeze. Reintroductions to the Grand Canyon and Mexican Baja California have subsequently been undertaken to avoid problems associated with proximity to humans. These birds are provided with supplementary feeding. The population into which birds are reintroduced is the one to which they are, on average, least related. As of July 2007, the population consisted of 306 individuals, 162 captive birds, 71 wild birds in California, 57 in Arizona and 16 in Baja.



California condor



**Fig. 20.7** Growth in number of the California condor populations in captivity and in the wild, including the number of individuals reintroduced.

## Guam rail

The Guam rail is a flightless, omnivorous bird restricted to the island of Guam in the Pacific Ocean. It numbered about 80 000 in the 1960s. However, it was extinct in the wild by 1986, as a consequence of predation from the brown tree snake that was inadvertently introduced during World War II. The Guam rail was saved from extinction by a captive breeding program based upon 21 founders (only 12 bred) rescued from the wild just prior to extinction. Haig *et al.* (1990) concluded that minimizing mean kinship was best of five alternative strategies for selecting breeders to produce young rails for reintroduction, and this was implemented. The overall goal of the captive breeding program is to produce about 100 rails each year for reintroduction

projects, while maintaining a population of 150 to 175 birds in zoos. Founder relationships were evaluated using DNA fingerprints and resulted in six individuals being placed in two different sib groups (Haig *et al.* [1994](#)). The captive breeding program has been highly successful. Further, 384 rails have been released in 1989–2000 on nearby Rota Island, where the brown tree snake is absent (S. Medina, pers. comm.). After initial difficulties, released birds are now surviving and breeding. In 1998, a reintroduction program was commenced in Guam, where birds were put into an enclosure designed to exclude brown tree snakes. Reintroduced birds and their progeny on Rota and Guam now number almost 400.



Guam rail

## **Lord Howe Island woodhen**

The Lord Howe Island woodhen is considered by ecologists to be a classic case of captive breeding and reintroduction (Caughley & Gunn [1996](#)). The endangered population on Lord Howe Island, off the east coast of Australia, fell to 30 individuals mainly due to predation by introduced pigs (NSW NPWS [2002](#)). The only known three breeding pairs were captured and bred in enclosures on the island and feral pigs were exterminated. Following reintroduction of 82 birds over four years, the population increased to 220–230 and appeared to be stable, so that a downgrading of its local threat status was recommended. However, it subsequently declined to around 160 by 2002 and is listed as endangered (IUCN [2007](#)).



Lord Howe Island woodhen

Unfortunately the genetic management was poor. As only three pairs contributed to the captive breeding program, the total population will be much more inbred than prior to the program, and genetic variation is likely to be lost due to the distorted contributions of the available individuals. In hindsight, it would have been desirable to enhance the genetic base of the captive breeding program once it was known to be successful, i.e. by adding further fertile individuals to the captive breeding program. Unfortunately, no genetic data on the population have been reported, no remedial action was planned, and routine monitoring was stopped. Given the history of this species, it is likely to have heightened susceptibility to new diseases and to environmental catastrophes (Brook *et al.* [1997a](#)). Recently, its approved recovery plan recommended (a) that a captive population be founded off the island as insurance against catastrophes, (b) that captive breeding be instituted on the island if population numbers decline seriously, and (c) that improved quarantine controls be made over birds coming onto the island (NSW NPWS [2002](#)).

## **Mauna Kea silversword**

By the late 1970s, only a small remnant natural population of the Mauna Kea silversword plant remained in Hawaii, following grazing pressure by introduced ungulates. Since 1973, 450 plants have been outplanted to promote recovery of this population. However, genetic analysis using RAPDs indicates that all outplanted individuals are derived from only two maternal founder plants, resulting in a major reduction in genetic variation in the total population (Robichaux *et al.* [1997](#)). Since the silversword is self-incompatible, natural pollination of the outplanted individuals results in only a 20% seed set, while deliberate outcrosses to the remaining wild individuals

increases it to 60%. A remedial program to outcross the outplanted individuals to the remaining wild individuals is under way to broaden the genetic base of the population. This is a challenge because there are only 46 original adult plants remaining in the wild and they flower only once before dying. With a lifespan of up to 40 years, the chance of any plants flowering in any given year is very low.



Mauna Kea Silversword

## Summary

1. Captive populations provide a source of individuals to reintroduce and supplement wild populations of threatened species.
2. The success of reintroduction is jeopardized by genetic deterioration in captivity due primarily to inbreeding depression, loss of genetic variation and genetic adaptation to captivity.
3. Genetic adaptations to captivity are overwhelmingly deleterious when populations are reintroduced to the wild.
4. The optimum means for managing captive populations for reintroduction is to maintain them as small partially isolated sub-populations, with occasional migration, provided no populations go extinct. In addition, this management reduces costs and risk of disease transfer.
5. Individuals for reintroduction should have high genetic diversity and maximum reproduction fitness in the wild environment. However, the risks involved dictate that reintroduction programs should begin with genetically surplus individuals with many relatives in the

captive population, so that the captive population is not compromised.

6. The initial priority with reintroduced populations is to increase numbers.
7. Once successful, the wild population should be augmented until the full complement of genetic diversity is represented in the wild.
8. To date, reintroductions have a relatively low success rate.

## Further reading

Ballou *et al.* (2009) Review on genetic and demographic management of captive populations and reintroductions.

Falk *et al.* (1996) *Restoring Diversity*. Review of plant reintroductions, with several case studies.

Frankham (2008) Review on genetic adaptation to captivity and means for minimizing its deleterious effects on reintroductions.

Re-introduction Specialist Group of IUCN. Website with access to *Re-introduction News* and policy documents. [www.iucnsscrg.org](http://www.iucnsscrg.org)

Reisenbichler *et al.* (2003) Review of genetic issues in restoring fish populations.

Stanley Price (1989) *Animal Re-Introductions*. Documents the classic reintroduction of the Arabian oryx into Oman and reviews reintroductions.

## Software

PMx: Population analysis software for studbooks that includes functions to evaluate the effects on mean kinship of different choices of animals to breed and to reintroduce (Pollak *et al.* 2009).

VORTEX: Software that can model genetic management of captive populations and reintroductions (Lacy *et al.* 2005). [www.vortex9.org/vortex.html](http://www.vortex9.org/vortex.html)



## **Problems**

- 20.1** Genetic adaptation. What are the reasons for concerns about genetic adaptation to captivity in captive breeding and reintroduction programs?
- 20.2** Genetic adaptation. What determines the rate of genetic adaptation to captivity?
- 20.3** Reintroductions. What are the genetic issues in reintroduction programs?
- 20.4** Reintroductions. What is the impact of release of a genetically over-represented captive animal (one with a high mean kinship) on mean kinship and heterozygosity in the captive population?

## Chapter 21 Use of molecular genetics in forensics and to understand species biology

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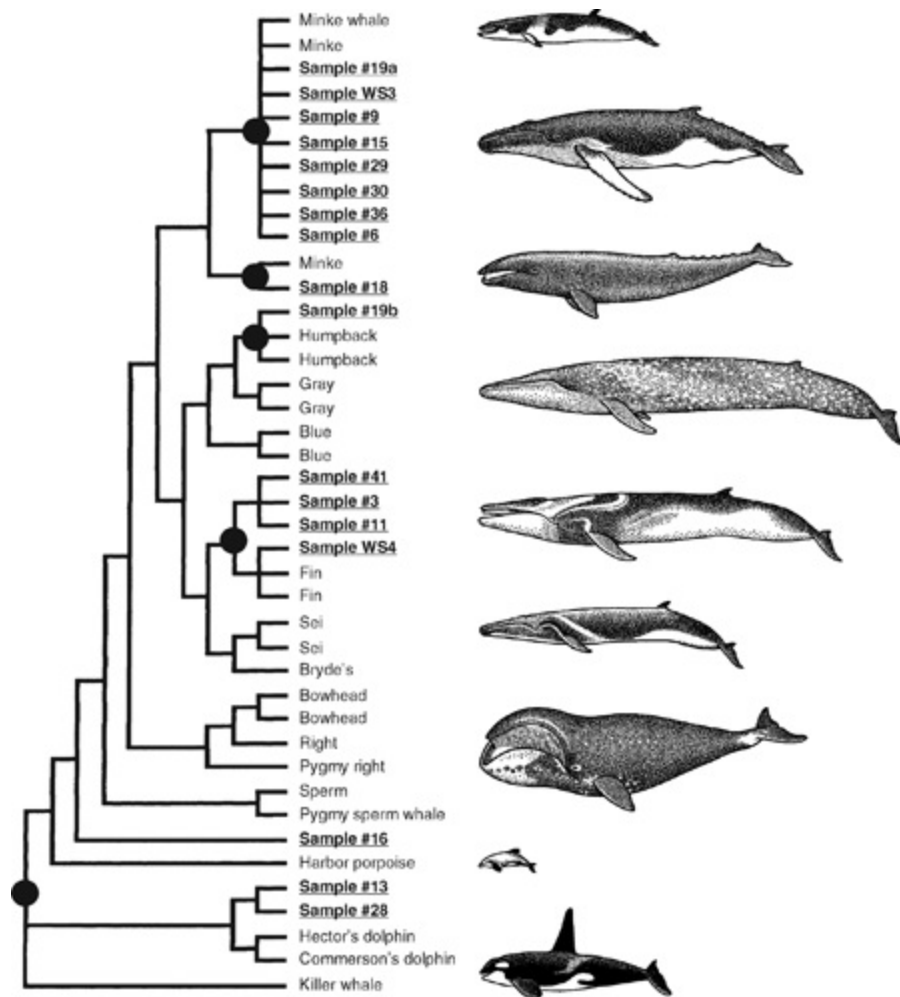
Molecular genetic analyses contribute to species conservation by aiding forensic detection of illegal hunting and by resolving important aspects of species biology. Coalescence and gene tree analyses provide useful tools for understanding many of these factors

### **Terms**

Allelic dropout, assignment test, biparental inbreeding, clade, forensics, haplotype network, molecular clock, phylogeography, selective sweep

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Whale forensics: mtDNA tree for whales and dolphins. Samples of whale products purchased in Japan are shown on the tree. Those for minke whales are legitimately sourced from legal 'scientific' whaling

(after Baker & Palumbi 1996)

## Forensics: detecting illegal hunting and collecting

PCR-based genetic markers can aid in detection of illegal hunting or collecting

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Poaching and illegal harvest threaten a wide variety of species, especially large cats, bears, elephants, rhinoceroses, parrots, whales and some plants. However, it is often difficult to obtain evidence to convict individuals illegally taking, or trading in, protected species. For example, a person carrying eggs suspected of belonging to threatened bird species was apprehended at an Australian airport, but avoided prosecution by squashing the eggs to avoid species being identified. Molecular genetic markers can now be used in such cases to identify the origin of biological material including eggs, feathers, hair, horns, ivory, meat, turtle shells and plant materials. The US Fish and Wildlife Service has established a forensics laboratory in Oregon to provide evidence in cases involving illegal imports, exports and hunting of endangered species. Similar analyses are being done in other countries.

An intriguing case of molecular forensics involved whale meat on sale in Japan and Korea ([Box 21.1](#)). Analyses of mtDNA established that some of the meat was not from minke whales, for which Japan engages in ‘scientific’ whaling, but from protected blue, humpback, fin and Bryde's whales. In addition, some ‘whale’ meat was from dolphin, porpoise, sheep and horse. Not only was illegal whaling suspected, but consumers were being misled. Similarly, PCR-based mtDNA analyses revealed that 23% of caviar being sold in New York was mislabelled (Birstein *et al.* [1998](#)). Many sturgeons are endangered due to over-fishing and habitat degradation. PCR-based DNA analyses have also been developed to detect tiger products in Asian medicines, shark species from dried fins, African elephants from ivory, rhinoceroses from horns, alligators from tanned hides and mammals from bushmeat (Teletchea *et al.* [2005](#)).

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### **Box 21.1 Detecting sale of meat from protected whales (after Baker & Palumbi 1996; Dizon et al. 2000)**

Following many years of commercial exploitation, the numbers of most whale species collapsed. The International Whaling Commission (IWC) instituted a global moratorium on commercial whaling in 1985–86. Some IWC members have continued to hunt a few whale species (primarily minke whales) for ‘scientific’ purposes and the whale meat is sold for human consumption. Suspicions arose that protected whale species were also being taken and marketed. Baker and Palumbi developed a system using mtDNA sequencing to reliably distinguish whale and dolphin species.

Samples of whale products were subsequently purchased in retail markets in Japan and Korea. To avoid the possibility of violating laws governing transport of endangered species products, Baker and Palumbi established a portable PCR laboratory in their hotel room and amplified mtDNA from the samples. The amplified DNA was subsequently sequenced in their laboratories in New Zealand and the USA.

Results from the initial 16 purchases are shown in the chapter frontispiece fitted into a mtDNA phylogenetic tree with known whale and dolphin samples. Nine samples near the top of the tree are indistinguishable from minke whales and represent meat acquired from legal ‘scientific’ whaling. However, sample #19b was from a protected humpback whale, while samples # 41, 3, 11 and WS4 were from protected fin whales. Consumers were being misled, as samples #16, 13 and 28 were from porpoise and dolphins.

Of 954 samples of ‘whale meat’ purchased in Japan and Korea up to 1999, 773 proved to be from whales, approximately 9% from protected blue, humpback, fin and Bryde’s whales. Non-whale samples included dolphins, porpoises, sheep and horses. The possibility that meat from protected species had been sourced from frozen stores, collected prior to bans on whaling, cannot be excluded, but this does not apply to fresh meat. Stricter controls over the distribution of ‘scientifically’ harvested

whale meat have been instituted and demands made that legally harvested whales and meat stockpiled prior to whaling bans be genotyped to monitor distribution.

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Forensic DNA analyses have also identified poaching of Eurasian badgers, Arabian oryx and sika deer (Marshall *et al.* 1999; Wu *et al.* 2005; Domingo-Roura *et al.* 2006). mtDNA and microsatellite DNA has been extracted from ivory and used to infer its geographic origin (Wasser *et al.* 2004). Subspecies of chimpanzees have been delineated, thus identifying the region where poaching was occurring, and where animals could be reinstated to the wild (Goldberg 1997).

## **Understanding a species' biology is critical to its conservation**

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Molecular genetic analyses help resolve many aspects of species' biology that are critical in conservation

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Critical aspects of the biology of many species are unknown, as details of life histories are often difficult and time-consuming to determine directly. Molecular genetic analyses can resolve paternities, define population structures, detect introgression from other species, evaluate sources of new founders for small endangered populations and indicate sites for reintroductions. Comparisons of DNA sequences may also be used to detect bottlenecks, migration patterns and the demographic histories of populations. For example, life history characteristics have been determined, and a

potential reintroduction site identified using microsatellites in northern hairy-nosed wombats ([Box 21.2](#)). In the threatened giant tortoises on the Galápagos Islands, mtDNA and microsatellite data have been used to resolve the source of the tortoises, patterns of migration, degree of genetic differentiation among island populations and populations within islands, to identify a new cryptic taxon and to define conservation units (Caccone *et al.* [1999](#); Beheregaray *et al.* [2002](#); Russello *et al.* [2005](#)).

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**Box 21.2 Censusing the critically endangered northern hairy-nosed wombat and inferring aspects of its biology following non-intrusive sampling of hair (Taylor *et al.* [1994](#), [2007](#); Beheregaray *et al.* [2000](#); Sloane *et al.* [2000](#); Banks *et al.* [2003](#))**

Northern hairy-nosed wombats are restricted to a tiny location in Queensland, Australia ([Chapter 11](#) frontispiece). They are nocturnal, fossorial, and difficult to study directly. Trapping has been used to estimate population size and sex-ratio, but is traumatic to the animals, risks possible injury or death, and is time-consuming and unreliable.

Using DNA from hair collected using adhesive tape on frames at the entrance to the wombat burrows, it was possible to genetically characterize the population for up to 20 microsatellite loci. Each individual in the population was identified and the population censused without disturbance. Individuals can be sexed by amplifying X- and Y-linked loci.

Microsatellite analysis of tissue from museum skins established that an extinct wombat population at Deniliquin was of the northern species, rather than the southern species currently found nearer to this location, thus identifying Deniliquin as a potential reintroduction site.

Microsatellites have also been used to infer aspects of the biology of the northern hairy-nosed wombats. Individuals of the same sex sharing burrows are often relatives, but males and females sharing burrows are

not close relatives. Dispersal patterns have also been deduced. The  $N_e/N$  ratio was estimated to be 0.1, based on loss of genetic diversity in comparison with the southern species.

**Table 21.1 Methods available for genetically characterizing individuals and populations and their applicability to each issue. Techniques with + can be used for the purpose specified, with several + indicating that the technique has higher utility, ? are cases where the technique is useful in only some cases, while – indicates that the technique is not useful in this context**

Issue	Morphology	Chromosomes	Allozymes	mtDNA	AFLP	DNA fingerprint	Microsatellites
Non-intrusive sampling	–	–	–	+++	++	–	+++
Forensics	–	–	+	+++	++	+++	+++
Population size	+	–	–	+++ <sup>f</sup>	+	?	++
Estimating $N_e$	–	–	++	++ <sup>f</sup>	–	?	+++
Demographic history	–	–	–	++	–	?	+
Detecting and dating bottlenecks	–	–	++	++ <sup>f</sup>	++	?	+++



Issue	Morphology	Chromosomes	Allozymes	mtDNA	AFLP	DNA	
						fingerprint	Microsatellites
Migration and gene flow	?	–	++	+ <sup>f</sup>	++	+++	+++
Individual identification and tracking	+	–	–	++	+	–	+++
Population structure	?	–	++	+?	++	++	+++
Phylogeography	–	–	–	+++	–	–	+++
Source populations to recover endangered species	+?	–	++	+	++	+++	+++
Introgression	+	+	++	+ <sup>f</sup>	++	+++	+++
Secondary contact	–	–	–	+++	–	+	+++
Taxonomic status	+	+++	++	++	+++	+++	+++
Sites for reintroduction	–	–	–	+	+	–	+++
Populations for reintroduction	+?	–	++	+	++	+++	+++
Reproductive systems	–	–	++	–	+	?	+++
Paternity	–	–	+	–	+	+++	+++
Founder relationships	–	–	?	–	+++	+++	++
Sources of new founders for endangered populations	+?	–	++	+	++	+++	+++
Sexing animals	?	+++	–	–	–	?+	+
Detecting disease	–	–	–	+++?	++	++	+
Diet	–	–	–	+++	++	++	++

<sup>f</sup> Can detect only female contributions.

The remainder of this chapter examines the methods used, and presents examples of practical issues that have been resolved. More advanced technical treatments can be found in Smith & Wayne (1996) and Zimmer & Roalson (2005).

Currently available methodologies and their applicability to the issues considered here are listed in Table 21.1. In general, DNA-based methods are suitable for most purposes and can be analysed following non-intrusive sampling and PCR amplification (Chapter 3).

## Coalescence and gene trees

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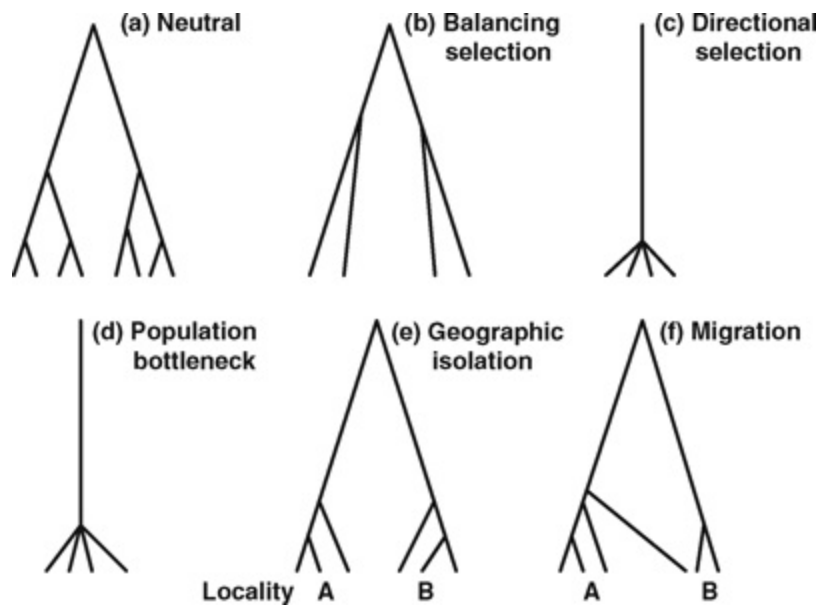
Alterations in coalescence patterns allow detection of selection, isolation among populations and changes in population size

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Differences in DNA sequences, gene tree structure and coalescence rates ([Chapter 11](#)) allow us to infer details of population structure and evolution that are not easily, or are less accurately, identified using other techniques. Analyses of gene trees, using coalescence analysis, have been used to:

- estimate effective population sizes (using selectively neutral sequences)
- measure neutral mutation rates
- infer selection and determine its form
- determine migration events and measure migration rates
- determine phylogenetic relationships among geographically separated populations (and compare these among species to determine whether patterns are concordant)
- detect secondary contact of diverged populations
- estimate divergence times among populations
- determine demographic history
- detect recombination in disease organisms
- reconstruct the origins and history of disease epidemics.

The structure of gene trees and patterns of coalescence are strongly influenced by deviations from selective neutrality and random mating ([Fig. 21.1](#)). Directional selection reduces the coalescence time, while balancing selection increases it, compared to the expectation from genetic drift alone. Coalescence of alleles in the MHC or SI loci (that are subject to balancing selection) often extend back prior to speciation events (trans-species polymorphism) ([Chapter 9](#)).



**Fig. 21.1** Gene trees showing coalescence patterns for (a) neutrality, (b) balancing selection, (c) directional selection (selective sweep), (d) population bottleneck, (e) geographic isolation and (f) migration.

After long periods of isolation and lack of gene flow, populations are deeply divided (Fig. 21.1e). Migration results in alleles characteristic of one geographic region being found in another region (Slatkin & Maddison 1989) (Fig. 21.1f). Fluctuations in population size and population bottlenecks foreshorten coalescence time (Fig. 21.1d). Mutations generate sequence differences, slowing coalescence times.

When patterns are similar, such as those for directional selection to fixation (**selective sweeps**) and population bottlenecks, additional information is required to resolve the cause. For example, information on multiple unlinked loci allows discrimination of selective sweeps and bottlenecks (Fig. 21.1c and d); population bottlenecks affect all loci in a similar manner, while a selective sweep affects only a specific locus and surrounding regions (Chapter 10).

Most coalescent analyses to date have used mtDNA data, as recombination is essentially absent, inheritance is typically maternal and mtDNA has a lower  $N_e$  and a higher mutation rate than nuclear loci. Consequently, it can detect effects over shorter time spans than nuclear loci. Nuclear DNA

sequences have recently begun to be used in coalescence studies. Further details of analyses of gene trees, coalescence and phylogeographic patterns are given in Rosenberg & Nordborg (2002), Avise (2004) and Wakeley (2007).

## Population size and demographic history

### Population size

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Minimum estimates of population size can be obtained from the number of unique multilocus genotypes

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It is difficult to directly estimate population sizes in nocturnal, fossorial, rare and shy animal species and direct estimates from sighting and trapping may be expensive to obtain. Based on collection of hair or faeces, followed by DNA extraction and PCR amplification of microsatellites, it is possible to obtain estimates of population size. This has been done for several threatened species, including northern hairy-nosed wombats, brush-tailed rock wallabies and giant pandas (Banks *et al.* 2003; Piggott *et al.* 2006; Zhan *et al.* 2006). Costs of censusing brown bears using non-invasive genetic methods were lower than for one based on observations of bears from a helicopter (Solberg *et al.* 2006). DNA-based censusing has revealed that the number of minke whales in the South Korean whale meat market was almost double the official figures, a finding that has serious implications regarding the viability of the North Pacific minke whale populations (Baker *et al.* 2007). This latter method is also suitable for estimating numbers in the bushmeat trade in Africa and elsewhere.

DNA-based censusing is sensitive to genotyping errors. With low-quality

DNA, alleles may not always amplify (**allelic dropout**) and artefactual PCR products may appear (false alleles), resulting in genotyping errors of about 2% for a single genotyping (Bellemain *et al.* 2005). Replicate genotyping of samples is used to overcome these problems and a statistical method has been devised to jointly estimate the rate of genotyping errors and the population size from the data (Kalinowski *et al.* 2006).

Census sizes are typically inferred from genotype data using either capture–mark–recapture or rarefaction analyses. Rarefaction methods calculate the population size as the asymptote of the relationship between the cumulative number of genotypes and the number of samples typed. Capture–mark–recapture estimates were considered more credible than those from rarefaction in a study of a brown bear population in Sweden (Bellemain *et al.* 2005).

Scat (faeces) counts have been used to estimate population sizes for many species (e.g. bears and coyotes), but these cannot be applied where more than one species with similar scats cohabits an area. DNA analyses have been used to authenticate bear scats in Europe (Höss *et al.* 1992).

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PCR-based genetic markers can be used to identify species when using faeces to estimate population size

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## Demographic history

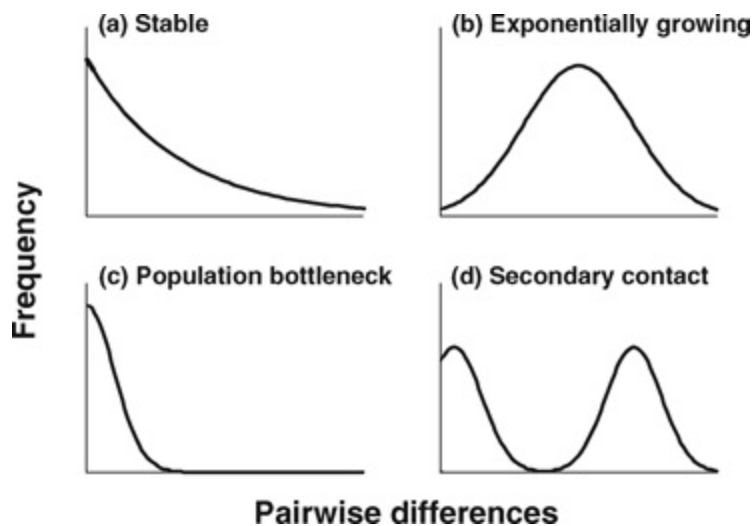
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The distribution of sequence differences between pairs of alleles can be

used to distinguish between stable, growing and bottlenecked populations

The distribution of the number of sequence differences between pairs of alleles (a **‘mismatch’ analysis**) has characteristic shapes for populations with different demographic histories (Fig. 21.2). Stable populations yield geometric distributions (Fig. 21.2a), while exponential growth is expected to generate a smooth unimodal distribution (Fig. 21.2b). Bottlenecks yield either a distribution close to zero, or a bimodal distribution, depending on whether the bottleneck reduced genetic diversity, or completely removed it (Fig. 21.2c). Secondary contact of populations following long isolation yields a bimodal distribution (Fig. 21.2d). Humans exhibit a unimodal distribution characteristic of exponential growth, consistent with known human history.

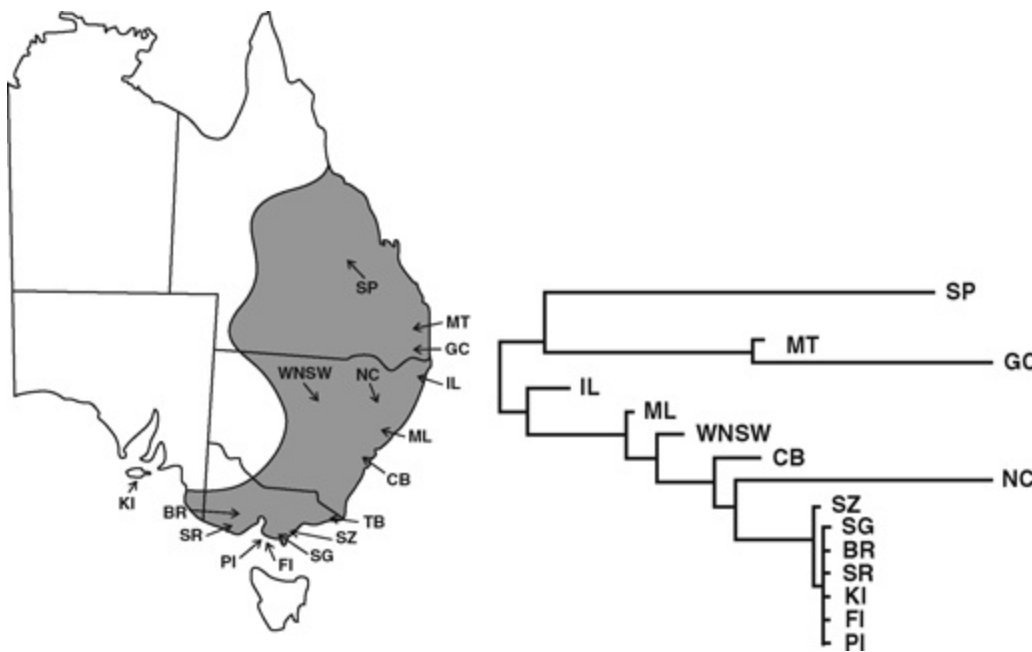


**Fig. 21.2** Distributions of pairwise sequence differences between alleles in populations with different histories (after Avise 2000). (a) Population with stable size, (b) population showing exponential growth, (c) population subject to a recent bottleneck and (d) secondary contact and fusion.

## Characterizing and dating bottlenecks

Signals of past population bottlenecks can be detected using molecular genetic analyses

Koalas exhibit signals of a population bottleneck in their mtDNA sequences, together with isolation-by-distance (Fig. 21.3). Most populations from Victoria and South Australia are indistinguishable, in contrast to the diversity among populations further north. This accords with their history (Box 17.3). Even when there are no samples of the pre-bottleneck population, bottlenecks can often be identified using tests based on information from multiple microsatellite loci (Luikart & Cornuet 1998).



**Fig. 21.3** Gene tree for koala populations, based on mtDNA sequence divergence (after Houlden *et al.* 1998). *Populations from Victoria and South Australia (bottom), derived substantially from a bottlenecked island population, are essentially indistinguishable. The remaining populations generally show their closest affinities with geographically adjacent populations, as expected with isolation-by-distance.*

Bottleneck effects have been measured by comparing microsatellite genetic diversity from the current populations with that from museum specimens for Mauritius kestrels and the Illinois population of greater prairie chickens (Boxes 8.1 and 11.2). A genetic signature of a recent population collapse in orangutans has been detected in microsatellite data, based on loss or excess of rare alleles, and models of the allelic frequency distributions (Goosens *et al.* 2006). The Galápagos tortoise sub-species found around the Alcedo Volcano on the island of Isabella has a low level of genetic diversity and genetic analyses revealed a signal of a population size bottleneck dating to the time of a known explosive volcanic eruption (Beheregaray *et al.* 2003).



Koalas

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The size and duration of bottlenecks can be inferred from loss of genetic diversity

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Loss of genetic diversity can indicate the size and duration of bottlenecks. For example, mathematical modelling of the loss of mtDNA genetic diversity and population growth trajectories in the northern elephant seal indicates that it probably sank to a single generation bottleneck of 10–20 effective females (Box 21.3). The 13 species of Darwin's finches on the Galápagos Islands



were thought to have diverged from the progeny of a single ancestral pair. However, a comparison of current MHC sequence diversity in Darwin's finches with simulations of models with founder effects and overdominant selection, indicated that the finches probably speciated from a founding group of at least 30 individuals (Vincek *et al.* 1997). Methods for inferring bottleneck size are reviewed by Leblois & Slatkin (2007).

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### **Box 21.3 Estimating bottleneck size in northern elephant seals (after Hedrick 1995a; Hoelzel 1999)**

Northern elephant seals underwent a bottleneck due to hunting, but the actual size and duration of the bottleneck is unknown. The last major hunt occurred in 1884. Subsequently, the population expanded to 350 in 1922, to 15 000 in 1960 and to ~150 000 when the study was done. Only two mtDNA haplotypes occur in post-bottleneck northern elephant seals, compared to 23 in related southern elephant seals. No allozyme variation was found in the northern elephant seal, while the average heterozygosity is 0.03 in southern elephant seals. The expected loss of mtDNA diversity is:

$$H_t = H_0 \prod_{i=1}^t \left(1 - \frac{1}{N_{efi}}\right)$$

where  $N_{efi}$  is the effective number of females in generation  $i$  and  $t$  is the number of generations from the beginning of the bottleneck until the population was genotyped. This is similar to Equation 11.2, but  $N_{ef}$  replaces  $2N_e$ , as mtDNA is maternally inherited (Table 11.1). The mtDNA diversity in southern elephant seals is 0.980 (assumed to represent  $H_0$ ), while that in northern elephant seals is 0.409 ( $H_t$ ).

Many combinations of bottleneck sizes and durations can fit these data, but only a limited range will allow realistic growth in population numbers. A single-generation bottleneck would require the effective number of females to be

$$\frac{H_1}{H_0} = \left(1 - \frac{1}{N_{ef}}\right) = \frac{0.409}{0.980} = 0.417$$

yielding

$$N_{ef} = 1.7$$

However, this is not compatible with the observed population growth, which requires a minimum of about 12 females. Further, additional genetic diversity is lost during approximately 14 generations between the bottleneck and 1960.

The combinations of parameters that best fitted both the loss of mtDNA diversity and the changes in population size were (i) a single-generation female bottleneck of 12.4 with  $N_{ef}/N_f = 0.25$  ( $N_{ef} = 3.1$ ), or (ii) a bottleneck of three generations with 44 females and  $N_{ef}/N_f = 0.125$  ( $N_{ef} = 5.5$ ). Hoelzel (1999) reached similar conclusions, based on detailed computer simulations. This bottleneck is not sufficient to account for complete absence of allozyme variation, but can be accounted for if the observed harem mating system is included in the model (Hoelzel 1999).

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Comparison of mtDNA from modern and ancient DNA in the nene in Hawaii revealed that the major decline in genetic diversity did not result from Western colonization, but from earlier Polynesian settlement (Paxinos *et al.* 2001). Modelling of the type illustrated in Box 21.3 indicates that the population probably declined to fewer than 10 females over 50–100 generations.

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The timing of bottlenecks can be inferred from genetic data

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It has been hypothesized that the cheetah lost substantial genetic diversity due to a population size bottleneck. The presumed bottleneck was estimated to have occurred ~10 000 years ago, based on comparisons of current genetic diversity and predicted rates of regeneration for allozymes (low mutation rate), with mtDNA, DNA fingerprints and microsatellites (higher mutation rates) (O'Brien 1994). However, there are alternative interpretations in this case (Hedrick 1996).

## Gene flow and population structure

### Secondary contact between populations

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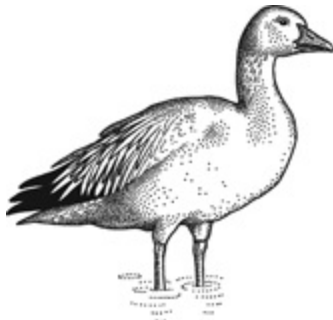
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Secondary contact between populations gives a bimodal distribution of pairwise sequence differences among individuals

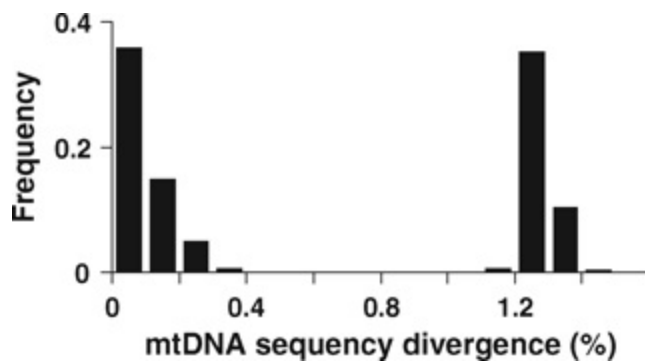
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Two distinct groups of mtDNA haplotypes (**clades**) are present in each surveyed snow goose rookery in the Canadian and Russia Arctic (Fig. 21.4). The rookeries have been established by migrants, presumably from two separate, diverged refuge populations, as (a) both mtDNA clades interbreed freely within any rookery, (b) the rookery sites occur in glaciated regions that were uninhabitable as recently as 5000–10 000 years ago, and (c) current populations are huge, so the distribution of haplotypes is not due to a recent drift effect.



Snow goose



**Fig. 21.4** Bimodal distribution of pairwise differences in mtDNA RFLPs for snow geese (after Avise 2000) indicating secondary contact.

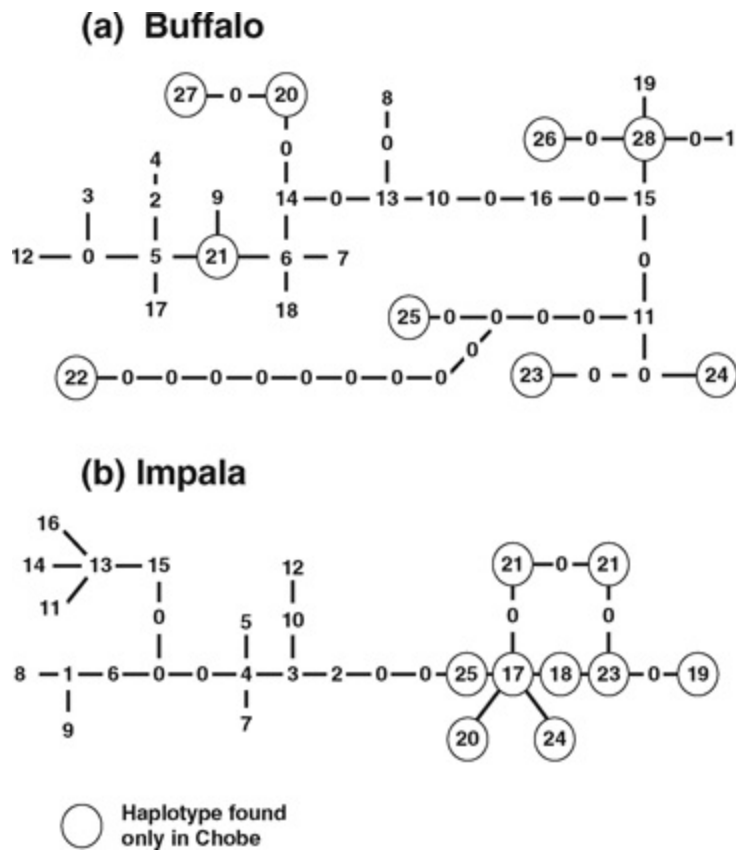
## Population structure

Analyses with genetic markers are used to determine population structure

Genetic management recommendations vary significantly depending on population structure (Chapters 14 and 17). The delineation of population structure is usually only possible using genetic data (reviewed by Hey & Machado 2003).

The degree of population differentiation can be determined using  $F_{ST}$  and

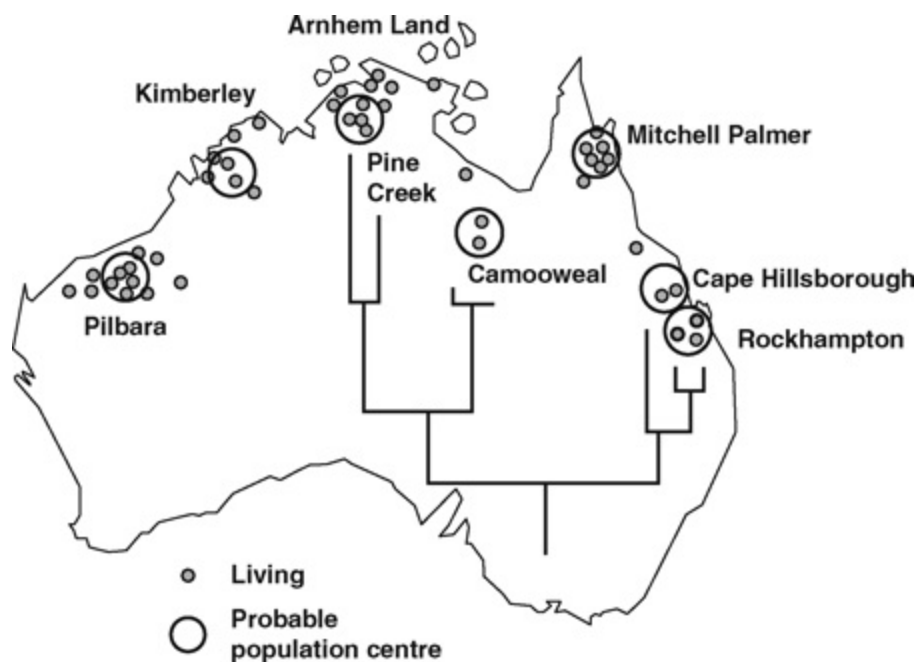
related measures for polymorphic genetic markers ([Chapter 14](#)). More powerful and informative analyses are possible by mapping the sequences of different alleles onto geographic locations ([Templeton 1998](#)). The cause of genetic differentiation, restricted gene flow, past fragmentation or range expansion can then be determined. East African populations of buffalo and impala show similar  $F_{ST}$  values of 0.08 and 0.10. However, the distribution of mtDNA haplotypes over geographic locations is entirely different in the two species, as shown in the **haplotype networks** in [Fig. 21.5](#). The distribution of Chobe haplotypes (Chobe is the most isolated location) is random in buffalo, but tightly clustered in impala. Consequently, buffalo exhibit recurrent maternal genetic interchange between Chobe and more northerly populations. In contrast, impala have restricted female gene flow that either reflects isolation-by-distance or isolation of the Chobe population from the northern populations. These conclusions were reached using nested clade analysis, but there is now controversy concerning the reliability of this widely used method ([Petit 2008](#); [Templeton 2008](#)).



**Fig. 21.5** Mitochondrial DNA haplotype networks for buffalo and impala

(after Templeton 1998). Each line in the network represents a single mutational change. '0' indicates a node in the network that was absent in the sample. Haplotype numbers are those given in the original reference. *Chobe haplotypes, from the most isolated and southerly location, are tightly clustered for impala but interspersed throughout the buffalo network.*

Female population structure can be revealed using mtDNA or cpDNA (plants). Mitochondrial DNA analysis revealed that the vulnerable ghost bat populations in Australia exhibit marked differentiation among colonies, with no mtDNA haplotypes being shared among colonies (Fig. 21.6). Microsatellite analyses also revealed substantial differentiation between populations indicating little if any gene flow. Consequently, extinct colonies are unlikely to be recolonized by natural migration.



**Fig. 21.6** Population structure of ghost bat populations revealed by mtDNA (after Moritz *et al.* 1996 © Oxford University Press). This species lives in caves and abandoned mines and was once widespread, but has now contracted northward. *There is clear differentiation among populations. No mtDNA haplotypes were shared among populations.*

Studies of the long-finned pilot whale revealed a pod structure consisting

of single extended female lines, often containing more than 100 individuals (Amos *et al.* 1993). Neither sex of offspring disperses, but there was not significant inbreeding, as determined using nuclear genetic markers. This apparent contradiction was resolved when members of different pods were observed to mate when they encountered each other, thereby minimizing differentiation for nuclear loci.

## Dispersal and gene flow

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Dispersal rates can be inferred from genetic differentiation among populations

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Dispersal rates are difficult to study by direct observation, as rates may be low and long-distance dispersal too rare to measure with precision. An increasing number of studies use genetic markers to infer dispersal patterns. With no dispersal, mating will be non-random, resulting in a deficiency of heterozygotes (Table 14.2). For example, mice maintain territories within barns and do not mate at random, resulting in overall deficiencies of heterozygotes compared to Hardy–Weinberg expectations (Selander 1970).

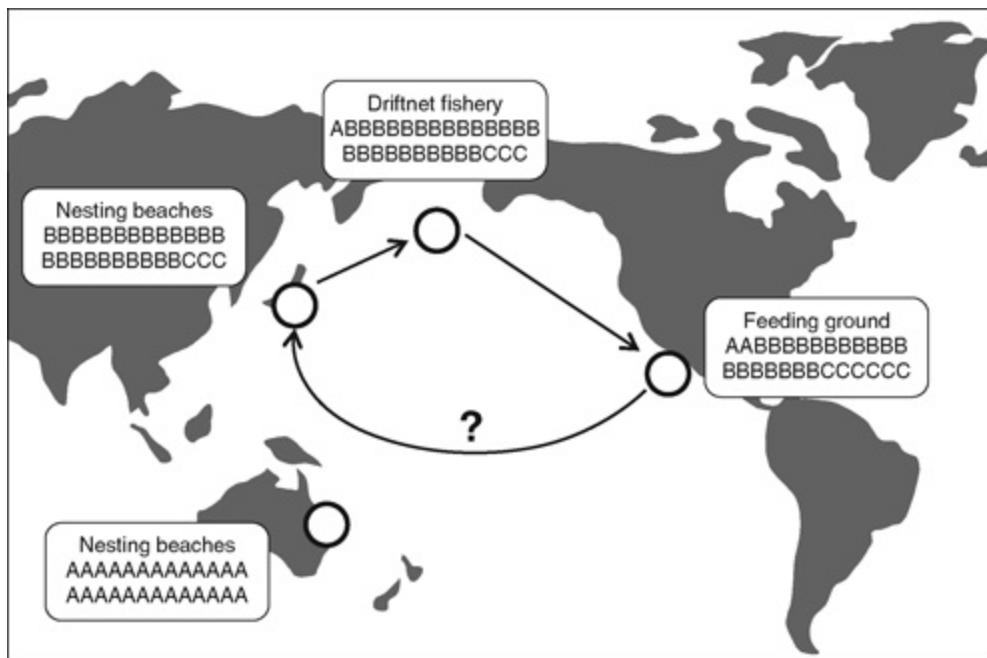
Genetic studies often reveal a picture of dispersal differing from that suggested by direct observations. For example, observations of territorial North American pikas indicated that they rarely dispersed, and they were thought to regularly mate with close relatives. In contrast, DNA fingerprinting studies revealed that close inbreeding was uncommon and that dispersal occurred over short, medium and long distances (Peacock 1997).

Based on mtDNA analyses, loggerhead turtle females show fidelity to nesting sites, but not to feeding grounds (Bowen & Karl 2007) (Fig. 21.7).

Similar studies have traced movement of endangered humpback whales (Baker & Palumbi 1996).



Loggerhead turtle



**Fig. 21.7** Dispersal in loggerhead turtles based on mtDNA haplotypes (after Bowen *et al.* 1995). A, B and C are different mtDNA haplotypes.

Y-specific DNA markers allow male-specific dispersal and differentiation to be assessed in a parallel manner (Tucker & Lundrigan 1996). Use of mtDNA and nuclear microsatellites has established that males, but not females disperse in great white sharks (Pardini *et al.* 2001).

## Detecting immigrants

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Individual immigrants can be identified from multilocus genotypes using assignment tests

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Based on its genotype, an individual can be assigned to the population with which it has the greatest similarity (**assignment tests**) (Luikart & England 1999). If it is assigned to a population other than the one in which it was collected, it is likely to be an immigrant (Chapter 14). For example, if all individuals in geographic areas A and B have genotypes  $A_1A_1B_1B_1C_1C_1D_1D_1$ , and  $A_2A_2B_2B_2C_2C_2D_2D_2$ , respectively, then an individual in region B with the former genotype must be a migrant. An identical principle applies when populations differ in frequencies at several loci, but the computations are more complex and assignments are expressed as probabilities rather than certainty. Assignment tests can also be used to detect hybridization and taxonomic relationships, for example with the red and Algonquin wolves (Fig. 16.1).

## Identification of populations for use in recovery of endangered sub-species

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Genetic markers can be used to identify the most closely related sub-species to use for crossing to recover a sub-species that has declined to a single individual

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We have previously considered the Norfolk Island boobook owl and the

dusky seaside sparrow. In the boobook owl, mtDNA analyses confirmed that the New Zealand birds chosen for crossing were from the most closely related population available (Example 16.2). In contrast, unsuccessful attempts were made to cross the last dusky seaside sparrow with a more distantly related sub-species, when subsequent genetic analyses revealed that a more closely related sub-species had been available (Avisé [2004](#)).

## Phylogeographic patterns across species

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Past geological or climatic events may affect many diverse species in a similar manner

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In a surprising number of cases, patterns of DNA sequence divergence across geographic regions are concordant for many species (**phylogeography**) (Avisé [2000](#)). For example, black sea bass, seaside sparrow, horseshoe crab, American oyster and tiger beetle on the Atlantic coast of the USA show mtDNA haplotypes distinct from those in the Gulf of Mexico. The two areas are separated by the Florida peninsula (Avisé [2000](#)). These distributions were not previously recognized, nor predicted from current landforms. Such patterns appear to reflect earlier separation of populations by major geological events, past climatic events or a change in habitat resulting from climatic change. Many other cases of concordant phylogeographic patterns in distinct taxa have been found in the USA, including freshwater fish in east-versus west-flowing rivers in the southeast, and terrestrial and freshwater tetrapods between southeast and northwest. Similarly, four species of birds and a reptile display major genetic differences between the rainforest regions north and south of Cairns in northeastern Australia. The differentiation is presumed to reflect past rainforest contractions and expansions that led to long periods of isolation between the two areas (Joseph *et al.* [1995](#)). Concordant patterns across distantly related species strengthen inferences that

may be only weakly supported for an individual species.

## Reintroduction and translocation

### Sites for reintroductions and translocations

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Potential reintroduction sites can be identified by PCR analyses of museum specimens collected from populations that are now extinct

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Reintroduction is a hazardous and expensive undertaking whose success is increased by selecting sites within the historical range of the species ([Chapter 20](#)). Characterization of an extinct population as belonging to an endangered species can indicate a suitable site for reintroduction or translocation ([Box 21.2](#)). DNA from sub-fossil bones revealed that Laysan ducks recently existed on a Hawaiian island where they are now extinct (Cooper *et al.* 1996). Consequently, this island may be a suitable site for re-establishment.

### Populations for reintroductions

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Evaluation of candidate populations for reintroduction can be made following genetic analyses

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A reintroduction program for the endangered shrub *Zieria prostrata* from a

restricted area on the east coast of Australia was abandoned when genetic analyses revealed that this apparently unique plant was closely related to a large extant population (Hogbin *et al.* [2000](#)).

## **Breeding systems, parentage, founder relationships and sexing**

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Genetic analyses can provide critical information on breeding systems, parentage, sex and founder relationships

As species with different forms of reproduction (asexual versus sexual, inbreeding versus outbreeding, etc.) require different management, it is vital to distinguish them ([Chapter 17](#)). Knowledge of parentage is critical to detect inbreeding, and to verify the accuracy of pedigrees used in genetic management. Correct assignment of sex is essential so that two individuals of the same sex are not paired. Founder relationships are important in managing captive populations so that loss of genetic diversity and inbreeding can be minimized. Genetic marker analyses can provide much of this critical information.

## **Breeding systems**

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Methods of reproduction and mating patterns can be resolved by typing mothers and offspring for multiple genetic loci

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Plants and corals have a diversity of mating systems from outbreeding to self-fertilization and clonal reproduction. Further, some species of fish, lizards, snails, slugs and insects are asexual, parthenogenetic or self-fertilizing.

[Table 21.2](#) illustrates determination of breeding systems from genotypes of mothers and offspring. If all offspring contain the same genotype as the mother then reproduction is asexual (including ameiotic parthenogenesis). Conversely, if offspring contain only alleles present in the mother, but have a diversity of genotypes then they are the result of self-fertilization. Offspring containing alleles not found in the mother are the result of outcrossing.

**Table 21.2** *Determination of breeding systems using genetic markers*

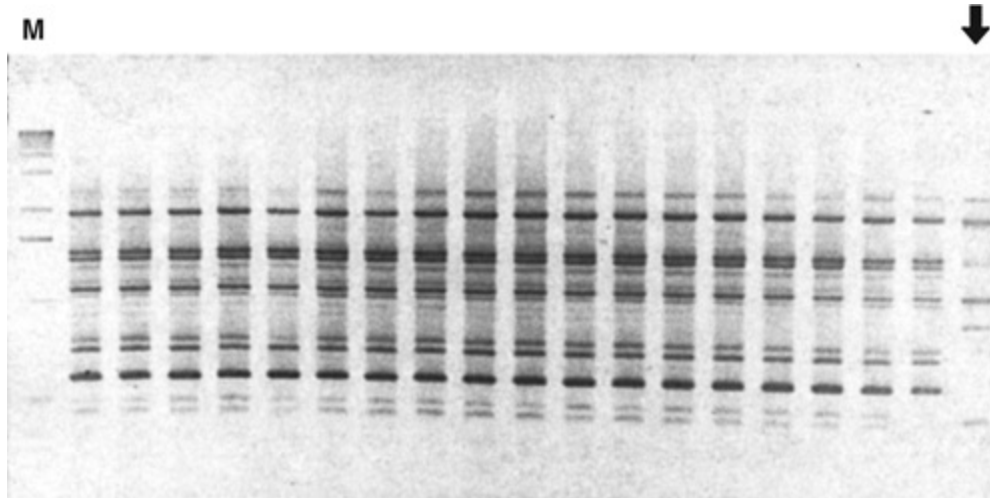
Breeding systems	Parent genotypes	Offspring genotypes
Asexual	AB	⇒ AB
Selfing	AB	⇒ AA, AB, BB
Outbreeding	AB × CD	⇒ AC, AD, BC, BD
Mixed selfing and outcrossing	AB × AB	⇒ AA, AB, BB
	AB × CD	⇒ AC, AD, BC, BD (heterozygote deficiency compared to outcrossing)

All individuals in one of two populations of the endangered Santa Cruz Island bush mallow plant in California were identical (indicating clonal reproduction) and different from individuals in the second population ([Fig. 21.8](#)). The endangered shrub *Haloragodendron lucasii* exists in a very restricted range in Sydney, Australia and comprises only seven clones among 53 plants, based on allozyme and RAPD genotypes (Hogbin *et al.* [2000](#)).

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In plants with mixed selfing and outcrossing, selfing rates can be

determined directly by typing maternal parents and progeny



**Fig. 21.8** Clonal reproduction in the endangered Santa Cruz Island bush mallow (from Fritsch & Rieseberg 1996). RAPD analyses on 18 different plants from the NS(II) population (lanes 2–19) and one individual from the NS(I) population (arrow). Lane 1 is a DNA size marker (M). *All 18 bushes from the NS(II) population are identical (clones) and different from the NS(I) plant.*

Table 21.3 illustrates the logic behind the method used to estimate selfing rates from maternal and progeny genotypes (Hedrick 2005a). Selfing of homozygous maternal plants results only in homozygous progeny, while outcrossing yields heterozygotes ( $H$ ) at a rate dependent upon the frequency of alleles not found in the homozygote ( $q$ ). Thus, the frequency of selfing ( $S$ ) is

$$S = 1 - \frac{H}{q} \quad (21.1)$$

Heterozygous maternal plants can also be used to obtain estimates of selfing rates (Brown 1989; Hedrick 2005a).

**Table 21.3** The proportion of progeny genotypes expected from a homozygous maternal genotype ( $A_1A_1$ ) and a heterozygous maternal genotype ( $A_1A_2$ ) as a result of self-fertilization ( $S$ ) and outcrossing ( $T$ ).  $p$  and  $q$  are the frequencies of alleles  $A_1$  and  $A_2$  in the population

Maternal genotype	Frequency of matings	Progeny genotypes		
		$A_1A_1$	$A_1A_2$	$A_2A_2$
$A_1A_1$	$S$	$S$		
	$T$	$Tp$	$Tq$	
$A_1A_2$	$S$	$\frac{1}{4} S$	$\frac{1}{2} S$	$\frac{1}{4} S$
	$T$	$\frac{1}{2} Tp$	$\frac{1}{2} T$	$\frac{1}{2} Tq$

Source: After Hedrick (2005a).

Hardy–Weinberg expectation can be used to estimate selfing rates. For example, the ratio of observed to expected heterozygosity ( $H_o/H_e$ ) is 0.68 in endangered round-leaf honeysuckle plants from Western Australia (Coates & Hamley 1999). Using Equation 12.2, the effective inbreeding coefficient is

$$F_e = 1 - \frac{H_o}{H_e} = 1 - 0.68 = 0.32$$

Selfing rates can be determined indirectly from the reduction in heterozygosity compared to Hardy–Weinberg expectations

The selfing rate can be determined from the inbreeding coefficient, as follows:

$$S = \frac{2F}{(1 + F)} \quad (21.2)$$

For round-leaf honeysuckle,  $S = 2 \times 0.32 / (1 + 0.32) = 0.48$ .

The most commonly used model to estimate selfing and outcrossing is the mixed mating model (Barrett & Kohn [1991](#)). This assumes that there are only two types of matings, self-fertilization and random mating. However, matings also occur among related individuals, such as full-sib, half-sib and cousin matings (**biparental inbreeding**). Consequently, the estimate of  $S$  is a measure of what the selfing rate would be, if all inbreeding was due to selfing.

Multilocus data provide more accurate estimates of true selfing rates. Further, in self-compatible plants, the difference between the mean of single-locus estimates and the multilocus estimate provides an estimate of biparental inbreeding. For example, individuals with only female flowers (male-sterile) in seven populations of *Bidens* spp. in Hawaii had an apparent ‘selfing’ rate of 15%, but all of this must be due to biparental inbreeding (Sun & Ganders [1988](#)). Similarly, the Pacific yew is dioecious (separate sexes), but has an  $F$  of 47%. Again, this must all be due to biparental inbreeding (El-Kassaby & Yanchuk [1994](#)). Barrett & Kohn ([1991](#)) and Hedrick ([2005a](#)) provide further details of methodology for estimating selfing and biparental inbreeding rates.

## Parentage

---

Multiple DNA markers can be used to assign paternity and maternity

---

Information on parentage is essential to study the impact of inbreeding, to verify pedigrees used in genetic management of threatened species and to determine the effective size of populations ([Chapters 11–13](#) and [19](#)).



Parentage cannot be determined from direct behavioural observations in species where females copulate with many males during their fertile periods, as occurs in chimpanzees. Nor can it be determined in secretive species.

If mother, offspring and putative fathers are genotyped for many loci (e.g. DNA fingerprints and multilocus microsatellites), positive paternity assignments can be made with high probabilities (Fleischer 1996). If a paternally derived allele in the offspring is not present in the suspected father, then that male can be excluded as a potential father (unless a new mutation has occurred). Table 21.4 illustrates parentage determinations in snow geese, based on 14 nuclear RFLP loci. At several loci, the genotype of gosling 4 cannot be derived from those of its putative (candidate) parents by Mendelian inheritance. Null alleles exist for some microsatellite loci and may lead to false parentage exclusions, but this problem can be alleviated by excluding such loci from parentage studies, or by requiring that parentage exclusions be based on more than one locus (Dakin & Avise 2004).

**Table 21.4 Parentage determinations in snow geese. Genotypes at 14 nuclear RFLP loci are given for putative parents and goslings in a family of snow geese (after Avise 2004). Gosling 4 does not match either of its putative parents. Alleles that cannot be inherited from its putative parents are shown in bold and genotypes that cannot be derived from putative parents are underlined**

	RFLP locus													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Putative father	22	22	23	12	11	11	14	22	12	12	12	12	22	22
Putative mother	22	22	22	11	11	11	13	12	22	11	11	12	12	12
Gosling 1	22	22	22	12	11	11	11	12	12	11	12	11	12	12
Gosling 2	22	22	22	11	11	11	34	22	22	12	12	12	22	22
Gosling 3	22	22	22	12	11	11	13	22	12	11	11	22	22	22
Gosling 4	<u>23</u>	22	<u>11</u>	11	11	11	<u>12</u>	12	12	11	11	11	<u>11</u>	<u>11</u>

Paternity determinations using microsatellites in captive chimpanzees revealed that the dominant male in the colony was responsible for siring

most, but not all, of the offspring (Houlden *et al.* [1997](#)). Consequently, the need to move animals among zoos to minimize inbreeding and loss of genetic diversity is greater than if many males contributed to paternity. Allozyme and microsatellite analyses of loggerhead turtle clutches established that females mated with several males, as offspring clutches contain more than two paternal alleles at some loci (Bowen & Karl [2007](#)).

Genetic marker analyses have often revealed unexpected mating patterns. Many birds, with presumed monogamous mating systems, have been shown to participate in extensive extra-pair copulations (see Fleischer [1996](#)). For example, splendid fairy wrens in Western Australia were reputed to have high rates of inbreeding, and no inbreeding depression. However, subsequent paternity analyses using allozymes revealed that 65% or more of progeny were fathered by males from outside the group (Ralls *et al.* [1986](#); Rowley *et al.* [1993](#)). Even in humans, genetic markers have revealed that 3.7% or more of children are not the offspring of their registered father (Bellis *et al.* [2005](#)).

As pedigrees are used extensively in the genetic management of captive populations, it is important to verify their accuracy. DNA fingerprinting in the critically endangered Waldrapp ibis identified five of 33 offspring whose pedigrees were incorrect and revealed an additional unrelated founder (Signer *et al.* [1994](#)). There also appear to be errors in the Bali starling, Arabian oryx and Przewalski's horse studbooks (Ashworth & Parkin [1992](#); Marshall *et al.* [1999](#); Bowling *et al.* [2003](#)).

## Reconstructing pedigrees

---

Pedigrees can be reconstructed from multilocus genotypes, but this is usually restricted to full and half-sibs

---

It is difficult or impossible to obtain pedigrees by direct observation for many wild species and even for multi-male multi-female breeding groups in some captive species. However, multilocus genotypic information can be used to reconstruct pedigrees for management purposes, or for estimating quantitative genetic variation in wild populations (Fernández & Toro 2006; Oliehoek *et al.* 2006). Pedigrees can usually be resolved only at the first and second order of relatedness (parent–offspring, full and half-sibs) due to the variability generated by Mendelian segregation within families. Genotyping errors, new mutations and missing genotypes make it even more difficult. Accuracy of pedigree reconstruction increases with the number of loci studied and the number of alleles per locus (Fernández & Toro 2006; Oliehoek *et al.* 2006). The correlation between actual and estimated kinship was over 90% for a pig population when 18 microsatellite loci were used (Fernández & Toro 2006).

Pedigree reconstruction based on microsatellite genotypes for nine loci in three populations of Atlantic salmon from eastern Canada revealed effective population sizes lower than census sizes due to unbalanced sex-ratios and variance in family sizes (Herbinger *et al.* 2006). The pedigree information allowed matings between relatives to be avoided and spawning from rare family lineages to be increased.

## Determining founder relationships

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Multilocus DNA markers can be used to delineate founder relationships

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Usually the relationships among the founders of breeding programs for endangered species are unknown. However, identifying related individuals is important in managing inbreeding and genetic diversity. Studies in the

California condor using DNA fingerprints revealed three clans of relatives amongst the 14 founders (Geyer *et al.* 1993). Similar studies have been conducted on Bali starlings, Guam rails, Micronesian kingfishers, Mauritius pink pigeons and Arabian oryx (Ashworth & Parkin 1992; Haig *et al.* 1994, 1995; Mace *et al.* 1996; Marshall *et al.* 1999).

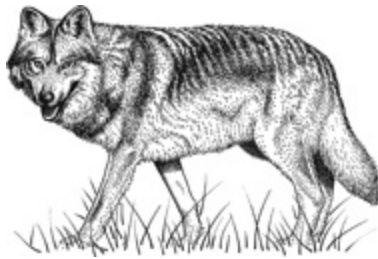
## Sources of new founders

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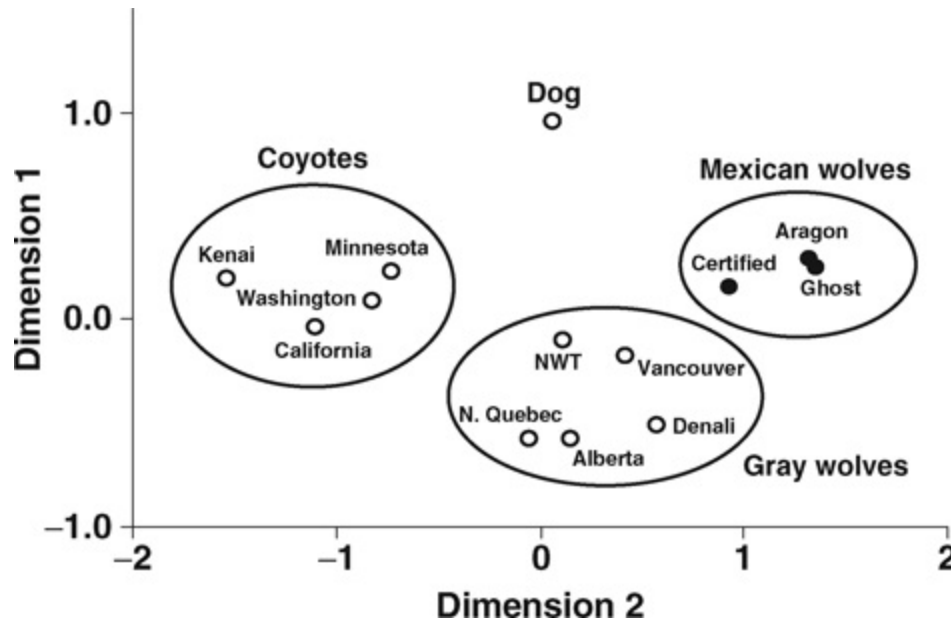
Where founder numbers are small, other potential founders can be examined, using genetic markers, to ensure that they belong to the correct species and are not affected by introgression

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When numbers of a threatened species are small, all potential founders should be used to establish captive breeding colonies. However, there may be uncertainties about the taxonomic identity of some potential founders. For example, the Mexican wolf is extinct in the wild and the single 'pure' Certified population was founded by only three or four animals. Two other populations existed, but it was unclear whether they had introgression from dogs, gray wolves or coyotes. Molecular genetic analyses (based on allozymes, mtDNA, DNA fingerprints and particularly microsatellites) established that all three populations of Mexican wolves were similar and pure, with no detectable introgression from dogs, gray wolves or coyotes (Fig. 21.9). These three populations have now been combined (Hedrick & Frederickson 2008). This study also determined that the Certified population had three, not four founders. In a similar manner, two potentially new founders for the US captive population of Speke's gazelle were shown to be unrelated to US animals and have been added to the captive population (Butler *et al.* 1994).



Mexican wolf



**Fig. 21.9** Are uncertified populations ‘pure’ Mexican wolves?

Multidimensional scaling of allele frequency data from 20 microsatellite loci typed in populations of Mexican wolves, coyotes, dogs and gray wolves (Hedrick *et al.* 1997). The Certified population consists of ‘pure’ Mexican wolves, while the Aragon and Ghost Ranch populations were of questionable status. Different coyote and gray wolf populations are indicated by state or province. *The Aragon and Ghost Ranch populations cluster with Certified as a group distinct from the other canids, indicating that all are ‘pure’ Mexican wolves.*

## Sexing animals

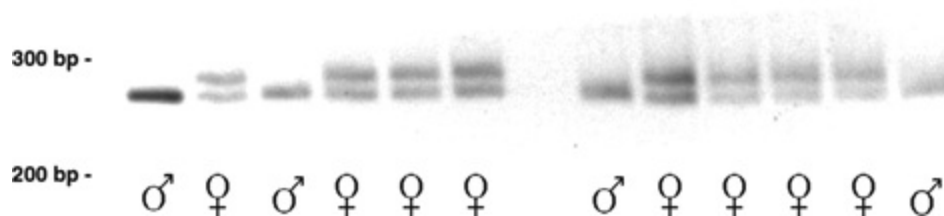
Birds and mammals can be sexed using genetic markers on the

heterogametic W and Y sex chromosomes

Males and females of many bird species are morphologically indistinguishable. Birds should be sexed prior to pairing, as several cases of ‘infertile’ pairs in zoos have turned out to be two birds of the same sex.

Some mammals are also difficult to sex, especially cetaceans (whales and dolphins) and secretive species. Therefore, it may not be possible to assign sex to individuals when collecting samples by skin biopsies, hair, etc.

Females and males cannot be morphologically distinguished in over 50% of bird species. Molecular sexing methods have been developed based on the fact that birds have ZZ male and ZW female sex-chromosome constitution. For example, PCR-based molecular sexing is possible for most bird species by amplifying an intron of the CHD locus which yields different sized fragments from the W and Z chromosomes (Kahn *et al.* 1998). A single band amplifies in ZZ males, while two different sized bands amplify in ZW females (Fig. 21.10).



**Fig. 21.10** Sexing of curlew birds using PCR amplification of an intron of the CHD locus with distinguishable sized fragments from the Z and W chromosomes (method of Kahn *et al.* 1998).

Molecular sexing is an important component in the program to recover the Norfolk Island boobook owl (Example 16.2). While the program had produced 12–13 individuals, of which seven were F<sub>2</sub>, only two pairs were

breeding. It was unclear whether this was due to hybrid sterility, unequal sex-ratio or individuals of one sex being immature. Molecular sexing revealed equal numbers of females and males, ruling out an unequal sex-ratio (Double & Olsen [1997](#)). Molecular sexing was used to avoid single-sex pairings in the recovery program for the critically endangered black stilt in New Zealand (Millar *et al.* [1997](#)). When applied to the critically endangered Taita thrush from Kenya this technique revealed a strongly distorted sex-ratio in one of three populations (only 10% females). Further, this study led to the identification of a morphological trait that can now be used to sex individuals visually.

Molecular sexing of extinct moas in New Zealand using ancient DNA methods plus mtDNA sequencing has revealed that morphological studies had incorrectly assigned sexually dimorphic males and females to different species (Bunce *et al.* [2003](#); Huynen *et al.* [2003](#)).

Since mammals typically have XX females and XY males, sex can be determined using molecular methods that detect distinguishable X and Y chromosome specific loci. For example, free-ranging Pyrenean brown bears were sexed from hair and faecal samples found in the field using a PCR amplification of a Y-specific locus (*SRY*) (Taberlet *et al.* [1997](#)). Similarly, molecular methods have been developed to sex cetacean skin biopsy samples (Bérubé & Pasbøll [1996](#)).

## Disease

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Molecular methods provide means for detecting and investigating the biology of disease organisms, and delineating the source of new diseases

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The disease status of animals is critical in identifying causes of population decline, and for checking candidates for translocation or reintroduction. PCR-based methods provide rapid, reliable and highly sensitive means for detecting disease organisms. For example, PCR has been used in studies of avian malaria in Hawaii, a major factor in the decline of endemic birds (Beadell *et al.* [2006](#)).

Gene trees based upon DNA sequences have been employed to determine the source of new animal and plant pathogens. HIV-1, one of the viruses that cause AIDS in humans, has been found to be most closely related to SIV from chimpanzees, while HIV-2 originated from sooty mangabeys (Sharp *et al.* [1996](#)). Similarly, a 1994 epidemic causing high mortality in Serengeti African lions was shown to be a zoonosis of canine distemper, presumed to have been contracted from local dogs (Roelke-Parker *et al.* [1996](#)). Recommendations were made to vaccinate local dogs against distemper to minimize the risk of repeat epidemics in lions and, especially, in other rarer carnivores.

## Diet

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Dietary items can be identified from gut contents or faeces by performing PCR with primers specific to suspected food items

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Diet is difficult to determine by direct observation in nocturnal and secretive species. In bears, the plant *Photinia* was identified as a food item, based on amplifying DNA from faeces using PCR primers specific to suspected food items (Höss *et al.* [1992](#)). The role of predators in causing the decline of a threatened species has also been assessed using PCR-based amplification and



genotyping. Microsatellite typing of stomach contents from glaucous gulls in Alaska revealed that they were preying on emperor geese, but not on threatened spectacled eiders (Scribner & Bowman [1998](#)).

## Aging and fitness from telomere lengths

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It may be possible to age individuals on the basis of the length of their telomeres

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It would be highly desirable to estimate age structure in endangered populations where the age of individuals is unknown (e.g. critically endangered kakapos in New Zealand). Population growth rates are quite different in populations consisting of senescent individuals, or juveniles, compared to those with primarily reproductive age individuals of both sexes. Telomeres are short tandem DNA repeats that stabilize the ends of eukaryotic chromosomes. Within species, telomere length typically shortens with age and this may allow the age of individuals to be estimated from a blood or tissue sample (Nakagawa *et al.* [2004](#)).

Telomere length may also provide an indirect measure of reproductive fitness. Age-corrected telomere lengths are associated with longevity in sand martin birds and with lifetime reproductive success in dunlins (Pauliny *et al.* [2006](#)). Further studies are required to determine whether this method is sufficiently reliable for use in conservation biology.

## Dating using molecular clocks

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Dates of evolutionary events can be estimated using the extent of genetic divergence and neutral mutation rates (the molecular clock)

---

The use of DNA or protein sequence divergence to date events using the **molecular clock** has been a major advance. Rates of nucleotide substitution are relatively constant over geological time ([Fig. 9.2](#)), presumably due to neutrality of most changes in amino acids and nucleotide sequences ([Penny 2005](#)). Molecular dating is conducted by creating a calibration curve of sequence divergence against time derived from the fossil record, and reading off dates for events with known molecular divergence, but unknown or poorly known times. Calibration curves are based on the assumption that the molecular clock ticks at a constant rate over time. Recent tests of the molecular clock against divergences with known geological dates generally reveal good agreement ([Benton & Ayala 2003](#); [Douzery \*et al.\* 2004](#); [Smith \*et al.\* 2006](#)).

However, the molecular clock does not tick at a completely invariant rate ([Pybus 2006](#)). Substitution rates can vary considerably between species for a wide range of taxa, including mammals, arthropods and vascular plants ([Thomas \*et al.\* 2006](#)). Different mutation rates, effective population sizes and generation lengths are among the variables associated with different rates of molecular evolution ([Nabholz \*et al.\* 2008](#)).

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The molecular clock does not tick at a constant rate across all species and time spans

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Recent clock methods that allow for rate variation in different organisms ('relaxed models') appear to perform much better than models assuming constant rates of molecular evolution (Drummond *et al.* 2006; Pybus 2006). With careful use, molecular clocks provide useful dates that are approximate, rather than precise, but their limitations must be kept in mind (Bandelt 2008).

## Summary

1. Molecular genetic analyses are invaluable in forensics and in resolving many aspects of species biology that are important in conservation.
2. Molecular genetic analyses have been used to
  - determine population size
  - infer demographic history
  - detect population bottlenecks
  - measure effective population sizes
  - determine reproductive and mating systems
  - establish parentage
  - sex individuals
  - determine founder relationships
  - identify the source of new founders
  - measure migration and gene flow
  - infer population structures
  - identify populations suitable for use in alleviating inbreeding depression
  - detect introgression
  - identify sites and populations suitable for reintroduction
  - document disease status and the source of new diseases, and
  - identify dietary items.
3. Gene trees and coalescence methods have added substantially to the analytical methods available to infer population processes of interest in the life histories of threatened species.

## Further reading

Avice (2000) *Phylogeography*. Book on phylogeography by the founder of the field.

Avice (2004) *Molecular Markers, Natural History and Evolution*. Discusses the use of molecular genetic techniques to understand species biology.

Beebe & Rowe (2007) *An Introduction to Molecular Ecology*. Textbook covering topics in this chapter.

Freeland (2005) *Molecular Ecology*. Textbook covering topics in this chapter.

Linacre (2009) *Forensic Science in Wildlife Investigations*. Textbook on wildlife forensics.

Zagorski (2006) Brief biography of Alec Jeffreys describing the discovery of DNA fingerprinting and the foundation of DNA-based forensics.

## Software

BOTTLENECK: Tests for population size bottlenecks (Piry *et al.* 1999). [www.montpellier.inra.fr/URLB/bottleneck/bottleneck.html](http://www.montpellier.inra.fr/URLB/bottleneck/bottleneck.html)

CAPTURE: Estimates population sizes from mark–recapture data (Otis *et al.* 1978). <http://welcome.warnercnr.colostate.edu/~gwhite/software.html>

CAPWIRE: Estimates population size from DNA-based capture–recapture data (Miller *et al.* 2005). [www.cnrhome.uidaho.edu/default.aspx?pid=69578/](http://www.cnrhome.uidaho.edu/default.aspx?pid=69578/)

CERVUS: Determines paternity from microsatellite data (Kalinowski *et al.* 2007). [www.fieldgenetics.com/pages/aboutCervus\\_Overview.jsp](http://www.fieldgenetics.com/pages/aboutCervus_Overview.jsp)

COLONY: Determines pedigrees from multilocus genotypic data (Wang 2004b). [www.zoo.cam.ac.uk/ioz/software.htm#COLONY](http://www.zoo.cam.ac.uk/ioz/software.htm#COLONY)

GIMLET: Estimates genotyping error rates, probability of identity, and parentage from multilocus genotypes (Valière 2002). <http://pbil.univ-lyon1.fr/software/Gimlet/gimlet.htm>

LAMARC: Estimates population sizes, exponential growth rates, migration rates and recombination rates from molecular data using coalescence (Kuhner 2006). <http://evolution.genetics.washington.edu/lamarc/lamarc.html>

MARK: Estimates census size from multilocus genotypes (White & Burnham 1999). [www.phidot.org/software/mark/](http://www.phidot.org/software/mark/)

MSVAR: Uses Bayesian methods to infer current and past population size from microsatellite allele frequencies. [www.rubic.rgd.ac.uk/~mab/](http://www.rubic.rgd.ac.uk/~mab/)

NEWPAT: Determines paternity from microsatellite data (Worthington Wilmer *et al.* 1999). [www.zoo.cam.ac.uk/zoostaff/amos/newpat.htm](http://www.zoo.cam.ac.uk/zoostaff/amos/newpat.htm)

PEDIGREE: Determines pedigrees from multilocus genotypic data (Herbinger *et al.* 2006). <http://herbinger.biology.dal.ca:5080/Pedigree/>

TCS: Determines haplotype networks (Clement *et al.* 2000). <http://darwin.uvigo.es/software/tcs.html>

## Problems

**21.1** Bottleneck. What proportion of the autosomal genetic variation is expected to remain in the Isle Royale population of gray wolves, compared to the mainland wolves, if it was founded from (a) one female mated to a single male? (b) one female mated to four males? (c) two mated pairs? (d) five pairs? Explain the basis of your calculations.

**21.2** Bottleneck. What proportion of the mainland mtDNA variation is expected in Isle Royale gray wolves with one, two or five female

founders?

**21.3 Bottleneck.** What constant female population size can explain the decline in mtDNA genetic diversity in the nene over 75 generations from  $H_0 = 0.80$  to  $H_{75} = 0.067$  (Paxinos *et al.* 2001)?

**21.4 Reproduction systems.** What are the methods of reproduction in the following cases?

- (a) Mother  $A_1A_2$ , 7 offspring  $A_1A_2$
- (b) Mother  $A_1A_2$ , offspring 5  $A_1A_1$ , 13  $A_1A_2$  and 6  $A_2A_2$
- (c) Mother  $A_1A_1$ , offspring  $A_1A_2$ ,  $A_1A_3$  and  $A_1A_2$
- (d) Mother  $A_1A_1$ , offspring  $A_1A_2$ ,  $A_1A_3$ ,  $A_1A_4$  and  $A_1A_5$
- (e) Mother  $A_1A_2$ , female offspring  $A_1A_3$ ,  $A_2A_3$ , male offspring  $A_1$  and  $A_2$ .

**21.5 Reproduction system.** What are all the possible explanations for all ~40 Wollemi pine plants having no genetic diversity at several hundred loci?

**21.6 Parentage determinations.** Determine whether each of the goslings in the snow goose family shown below is compatible with their putative parents. Diploid genotypes at 14 nuclear RFLP loci are given below (after Avise 2004). For example, the putative father has alleles 1 and 2 at locus A, 2 and 2 at locus B, etc.

Family 2	RFLP locus													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Putative father	1 2	2 2	2 4	1 1	1 1	1 1	1 1	1 2	1 1	1 1	1 1	1 2	1 1	1 2
Putative mother	2 2	2 2	1 2	1 1	2 2	1 1	1 2	1 2	2 2	1 1	1 1	1 1	2 2	1 2
Gosling 1	2 2	2 2	1 2	1 1	1 2	1 1	1 1	2 2	1 2	1 2	1 1	1 1	1 2	1 2
Gosling 2	2 2	2 2	2 4	1 1	1 2	1 1	1 2	1 1	1 2	1 1	1 1	1 1	2 2	1 1
Gosling 3	1 2	2 2	2 4	1 1	1 1	1 1	1 1	1 2	2 2	1 1	1 1	1 1	1 1	1 1
Gosling 4	2 3	2 2	2 2	1 1	1 1	1 1	2 2	1 1	1 2	1 1	1 1	1 1	1 1	2 2
Gosling 5	1 2	2 2	2 2	1 1	1 2	1 1	1 2	2 2	1 2	1 1	1 1	1 2	1 2	1 2

## **Chapter 22 The broader context: population viability analysis (PVA)**

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Population viability analysis assesses the combined impacts of both deterministic factors (habitat loss, over-exploitation, pollution and introduced species) and stochastic events (demographic, environmental and genetic stochasticity, and catastrophes) on extinction risk, and compares alternative management options in species recovery programs

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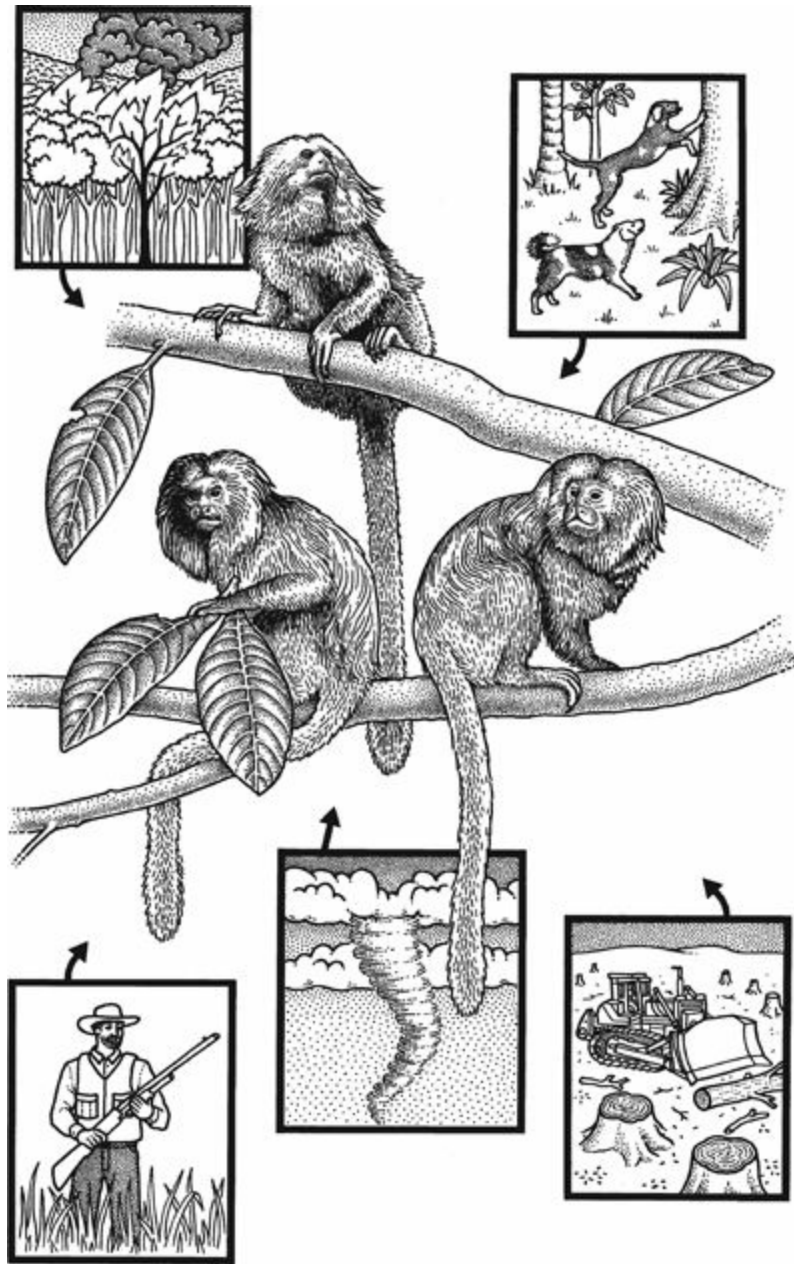
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### **Terms**

Demographic stochasticity, environmental stochasticity, genetic stochasticity, population and habitat viability assessment (PHVA), population viability analysis (PVA), sensitivity analysis

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‘It’s a hard world out there’: stylized scenario of the myriad risks faced by species in natural habitats



## What causes endangerment and extinction?

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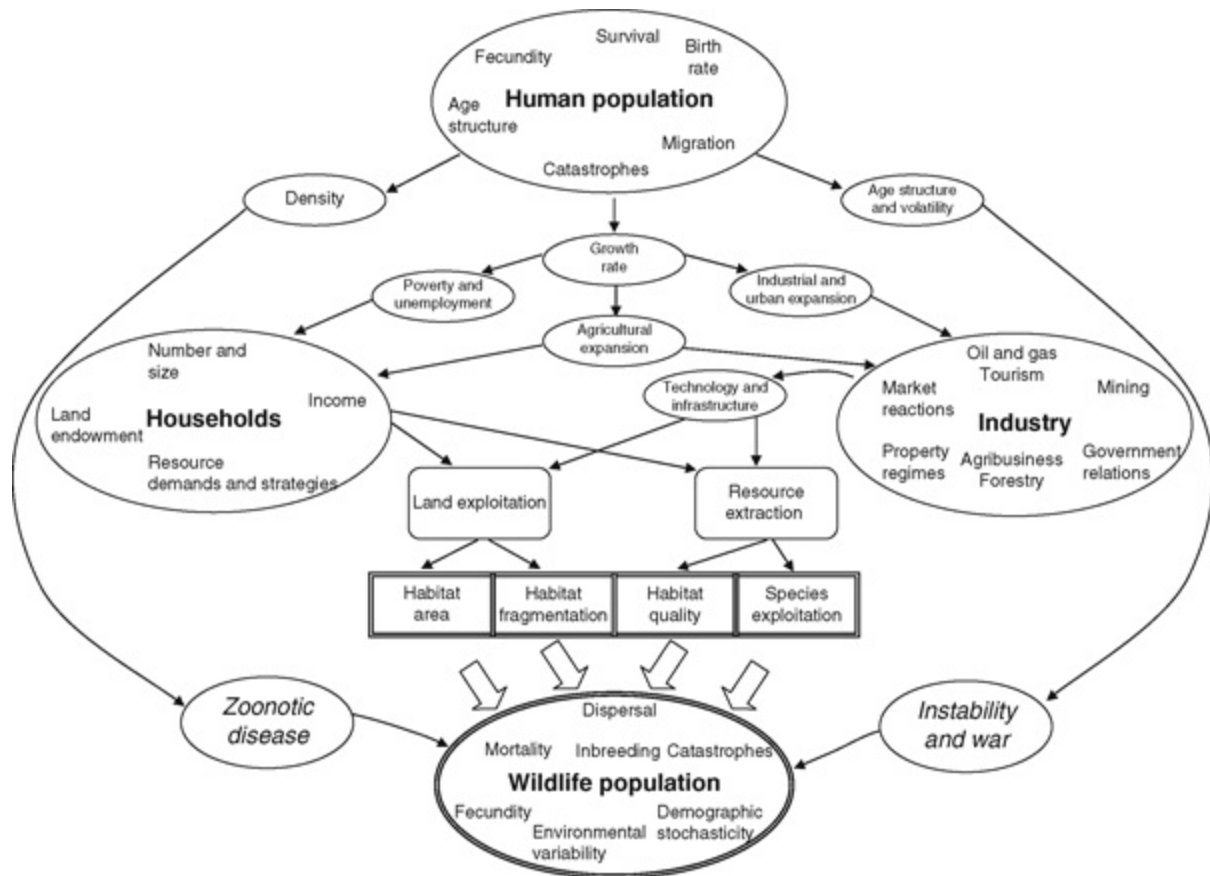
Population decline and extinction is usually due to the combined effects of deterministic and stochastic factors

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As genetic threats are only a part of the endangering processes faced by species in the wild, it is important to place them in the broader conservation context. Assessments of extinction risk are required so that populations can be categorized according to relative risk, and conservation priorities set. High-risk species are accorded legal protection in most countries and trade or movement of endangered species is restricted in countries that are signatories to the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES).

Until the 1990s, risk assessment was rather subjective, with listing of species as endangered being based largely on the persuasive powers of individuals. Subsequently, designations of degree of endangerment have been based on more objective, quantitative guidelines (Mace & Lande [1991](#); IUCN [2007](#)). This chapter is concerned with assessing extinction risk due to the combined impacts of deterministic and stochastic threats using computer modelling (population viability analysis: PVA). We discuss the factors important to population viability, how viability is assessed, and what has been learned using this approach. We also consider means for evaluating options for restoring threatened and endangered species.

Below we describe the deterministic and stochastic factors that may act, and interact, to drive populations to extinction. Many of these factors are listed in [Fig. 22.1](#).



**Fig. 22.1** Deterministic and stochastic factors affecting wildlife populations and their interactions (from CBSG).

## Deterministic factors

Many species have had their numbers reduced by deterministic factors associated directly or indirectly with human actions

Deterministic factors are those processes that have a consistent predictable direction and a relatively consistent magnitude. Most of the deterministic factors that cause the decline and extinction of species are directly, or

indirectly associated with human actions, namely:

- destruction of habitat for urban and agricultural development, etc.
- over-exploitation for commercial or recreational use
- inadvertent pollution and deliberate application of pesticides, herbicides, etc.
- exotic species introduced intentionally, or by accident (e.g. ballast water, international trade)
- combinations of the above factors.

While habitat loss is the single most important documented factor, in most cases several of the factors combine to drive species to extinction (UNEP 2007). Further, the deterministic factors reduce population sizes to the point where additional stochastic processes may become significant, leading to a positive feedback among the processes in the extinction vortex (Chapter 2).

## Stochastic factors

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Small populations face additional threats: demographic, environmental and genetic stochasticity and catastrophes

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Unlike deterministic factors, stochastic processes in small populations display large random components with effects varying in direction and magnitude over both time and space (Boyce *et al.* 2006). As briefly outlined in Chapter 1, there are four forms of stochasticity relevant to extinction risk in small populations:

- **demographic stochasticity** This describes the natural fluctuations in birth and death rates and sex-ratios that are independent of environmental effects (May 1973). Extinction can result if, by chance,

all individuals in a small population are sterile, or all of one sex. For example, the last six dusky seaside sparrows were all males, an event with a probability of  $(\frac{1}{2})^6$ ; nevertheless, this happened and the subspecies became extinct (Avice & Nelson 1989).

- **environmental stochasticity** Birth and death rates often fluctuate due to variation in the environment, such as those in rainfall, temperature, density of competitors, predators, food sources, etc. For example, birth and death rates are strongly affected by rainfall in red kangaroos and cycles in lynx populations in Canada appear to be driven by the North Atlantic Oscillation meteorological fluctuation (Caughley & Gunn 1996; Stenseth *et al.* 1999). Environmental variation influences all individuals in the population in a similar manner.
- **genetic stochasticity** This encompasses inbreeding depression, loss of genetic diversity, divergence of populations and accumulation of new deleterious mutations.
- **catastrophes** These are infrequent extreme environmental events such as cyclones, severe winters, fires, floods, volcanic eruptions and disease epidemics that may be the proximate cause of extinctions. For example, a hurricane caused a significant decline in population numbers of the endangered Puerto Rican parrot, while African lions in the Serengeti suffered high mortality due to canine distemper, and many frog species throughout the world are being decimated by a fungal disease (Lacy *et al.* 1990; Roelke-Parker *et al.* 1996; Mendelson *et al.* 2006; Pounds *et al.* 2006).

## Interactions of deterministic and stochastic factors

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Deterministic and stochastic factors operate in a feedback cycle termed the 'extinction vortex'

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The combined impacts of deterministic and stochastic factors are more damaging than the sum of their individual effects. Human pressures typically lead to small population sizes. This promotes inbreeding and consequent reductions in birth and survival rates. In turn, this causes further reductions in population size, increased demographic instability and a downward cycle to extinction, termed the ‘extinction vortex’ (Gilpin & Soulé 1986; Fig. 2.2). For example, the greater prairie chicken population in Illinois initially declined due to habitat loss and fragmentation, but genetic diversity was lost and the population became inbred, reducing fertility and hatchability in a feedback cycle (Westemeier *et al.* 1998). The population size continued to decline despite habitat restoration, leading to further inbreeding depression in reproductive rate. Extinction vortices have been observed in other wild vertebrate populations that have been lost (Fagan & Holmes 2006).

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Variation in population size due to demographic and environmental stochasticity and catastrophes reduces the effective population size and increases the rate of inbreeding

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As the effective population size is strongly dependent upon minimum population size, fluctuations in population size due to any cause affect  $N_e$ , inbreeding and extinction risk (Chapters 11–13). Thus, the combined impacts of demographic and environmental stochasticity and inbreeding depression reduce reproduction and survival more than expected from their individual effects (Brook *et al.* 2002; O’Grady *et al.* 2006).

As population declines typically result from both deterministic and stochastic factors, actions to recover threatened species must not only address the original causes of decline (usually deterministic factors), but also address the additional (later) stochastic threats. Identifying the most important factors

determining extinction risk can help identify possible remedial action for threatened populations.

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Extinction risk reflects the combined impacts and interactions of all deterministic and stochastic factors

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## **Predicting extinction probabilities: population viability analysis (PVA)**

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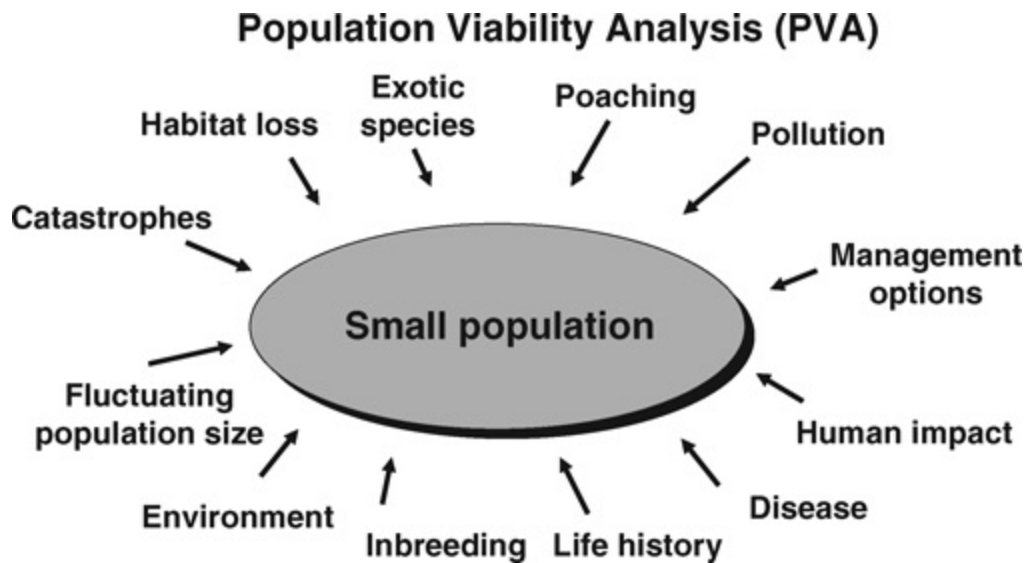
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Population viability analysis is used to predict the probability of extinction due to the combined effects of all deterministic and stochastic threats

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The process of combining the deterministic and stochastic risk factors to project the future fate of populations is called population viability analysis (Akçakaya & Sjögren-Gulve 2000) (Fig. 22.2). The outcomes have a large stochastic element and involve probabilities rather than certainties.



**Fig. 22.2** Population viability analysis (PVA) models the effects of different life history, environmental and threat factors on the population size and extinction risk of populations or species.

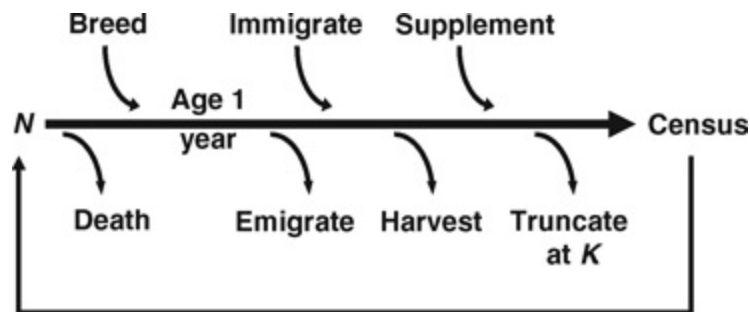
PVA arose out of the concept of minimum viable population size ([Chapter 15](#)), largely through the contributions of Shaffer ([1981](#)) and Gilpin & Soulé ([1986](#)). Hundreds of PVAs have now been completed for threatened species, especially by CBSG and the RAMAS group (Traill *et al.* [2007](#)). Detailed case histories for several PVAs are given later in this chapter.

PVAs are usually carried out by inputting, to a computer program, information on

- birth and survival rates and their variances
- number of populations
- population sizes
- habitat carrying capacities
- frequencies and effects of threats (e.g. catastrophes, hunting, etc.), and
- other details about species life history (e.g. susceptibility to inbreeding depression, rates of gene flow between populations, etc.).

The populations are then projected forward in time ([Fig. 22.3](#)). The concepts used in the computer simulation programs are based upon the

accumulated knowledge of more than 100 years of research into population demography, ecology and genetics. An example of the kind of input information required to run a PVA for the software package VORTEX (Lacy *et al.* 2005) is given for the golden lion tamarin in [Table 22.1](#).



**Fig. 22.3** Cycle of events in a typical PVA run as it progresses through generations (after Miller & Lacy 2005).

**Table 22.1** Example of input information for a population viability analysis run using the VORTEX software package (version 9.55) for a golden lion tamarin population (after Holst *et al.* 2006). EV is the standard deviation for that parameter attributed to environmental variation. Input variables are explained in the VORTEX software manual (Lacy *et al.* 2005).

## VORTEX

### Golden Lion Tamarin Vortex Input Data

This provides input data for the scenario WITH INBREEDING



Tab	Vortex Parameter	User Input
Scenario Settings	Project Name	Golden lion tamarin
	Scenario Name	Inbreeding
	No. Iterations	500
	Number Years	100
	Definition of Extinction	Only one sex
Species Description	Populations	1
	Inbreeding Depression?	yes
	Lethal Equivalents	4.07
	Percent Due to Recessive Lethals	50
	EV Concordance Repro and Survival?	yes
Labels and State Vars.	Number of Types of Catastrophes	2
	Ignore	
	Dispersal	Ignore
	Reproductive System	Mating System
		Monogamous
Reproductive Rates	Age First Offspring to Females	4
	Age First Offspring to Males	4
	Maximum Age Reproduction	16
	Maximum Number Progeny/year	5
	Sex Ratio at Birth	50
	Density Dependent Reproduction?	N
	% Adult Females breeding	73.0
	EV	9.4
	Number of offspring/F/year	Specify exact distribution
	Data: % females producing 1 Offspring	21.00
	Data: % females producing 2 Offspring	58.00
	Data: % females producing 3 Offspring	8.00
	Data: % females producing 4 Offspring	12.00
	Data: % females producing 5 Offspring	1.00
Mortality Rates	<b>Female</b> Mortality from age 0 to 1	32.6
	SD in 0 to 1 due to EV	14.1
	Annual Mortality ages 1 and 2	13.0*
	SD Ages 1 and 2	7.8*
	Annual Mortality ages 2 and 3	11.5*
	SD Ages 2 and 3	0*
	Annual Mortality ages 3 and 4	19*
	SD Ages 3 and 4	20.7*
	Annual Mortality adults	10.4*
	SD adults	7.7*

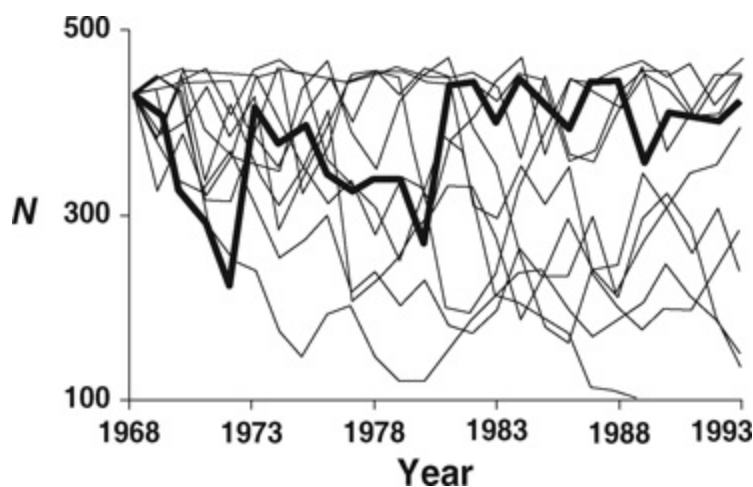
Tab	Vortex Parameter	User Input
Catastrophes	<b>Male</b> Mortality from age 0 to 1	31.9
	SD in 0 to 1 due to EV	14.0
	Annual Mortality ages 1 and 2	12.2*
	SD Ages 1 and 2	0*
	Annual Mortality ages 2 and 3	15.1*
	SD Ages 2 and 3	3.0*
	Annual Mortality ages 3 and 4	15.4*
	SD Ages 3 and 4	9.5*
	Annual Mortality adults	14.0*
	SD adults	20.9*
	Labels	Ignore
	<b>Catastrophe 1</b>	Disease
	Global/Local	Ignore
	Frequency %	1.0
	Severity–Reproduction	1.0
	Severity–Survival	0.5
	<b>Catastrophe 2</b>	Fire
	Global/Local	Ignore
	Frequency %	5.0
	Severity–Reproduction	1.0
	Severity–Survival	0.95
Mate Monopolization	% Males in the Breeding Pool	100.0
Initial Population Size	Start with	Stable Age Distribution
	Initial Population Size	347
Carrying Capacity	K	347
	SD in K due to EV	0.0
	Future change in K?	No – Unchecked
Harvest	Harvest?	No – Unchecked
Supplementation	Supplement?	No – Unchecked
Genetic Management	Ignore	No

\* In the published PVA, mortality rates were entered as functions of population size and carrying capacity, so results using above parameters will not match those in the PVA report (see Holst *et al.* 2006).

Replicate runs of PVA software using the same inputs give widely varying population trajectories as a consequence of stochastic variation

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Many replicate runs (typically 500–1000) are conducted for a given set of input data, as individual population projections differ amongst these stochastic simulations. While input data are identical for all runs, the results of each run vary due to random number generators being used to mimic the demographic, environmental and catastrophic stochasticity that actually occurs in the population. The variability added by the random number generator is dependent on the population size for that run, sampling theory, and the variance of rates provided by the input data (see VORTEX manual for details). For example, the variability among replicate runs for the Capricorn silvereye bird (all using identical input data) is shown in [Fig. 22.4](#).



**Fig. 22.4** Stochastic variability among replicate PVA runs in the projections for the Capricorn silvereye population from Heron Island in the Great Barrier Reef, Australia (from Brook & Kikkawa [1998](#)). The bold line is the average of all replicate runs.



Capricorn silvereye

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PVAs may be conducted using generic software packages, species-specific programs or simple  $r$ -models

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PVAs may be carried out using generic, off-the-shelf software packages (such as RAMAS GIS or VORTEX; Brook *et al.* [2000](#)), or customized programs written for a specific species. The generic packages differ in their characteristics, but all include demographic and environmental stochasticity. VORTEX is an individual-based program (i.e. it tracks the life of each individual in the population), while the RAMAS packages are matrix-based programs (that only track cohorts of individuals). RAMAS GIS and VORTEX are able to handle fragmented populations. VORTEX includes genetic factors, but functions can be included in RAMAS to include inbreeding effects.

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A major limitation of PVA is that insufficient life-history data exist for most species

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Limited life-history data exist for most threatened species, and full population viability analyses may therefore not be possible. A simplified version of PVA can be performed using stochastic  $r$ -models. These require only a time series of population size data to estimate the rate of population growth ( $r$ ) and its variance. Simple stochastic simulations can predict the future population size trajectory and provide reasonable projections (see

below). Example 22.1 illustrates a population trajectory in the Bay checkerspot butterfly, the estimation of  $r$  and its variance and the projection from stochastic  $r$ -model simulations.

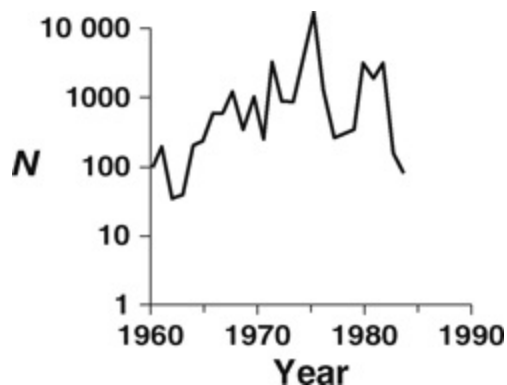
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### **Example 22.1 Use of an $r$ -model to project the future size of Bay checkerspot butterfly population in California (data from Foley 1994)**

The figure in the margin overleaf shows the population size trajectory for a Bay checkerspot butterfly population from 1960 to 1986. There are two populations, so we will consider one here and use the other as Problem 22.1.



Bay checkerspot butterfly



The rate of population growth,  $r$ , was obtained from the data as follows:

$$\frac{N_t}{N_{t-1}} = e^r = \lambda$$

and

$$\ln\left(\frac{N_t}{N_{t-1}}\right) = r$$

For example, the numbers in the first two generations were 90 and 175. From these, we obtain our first estimate of  $r$ , as follows:

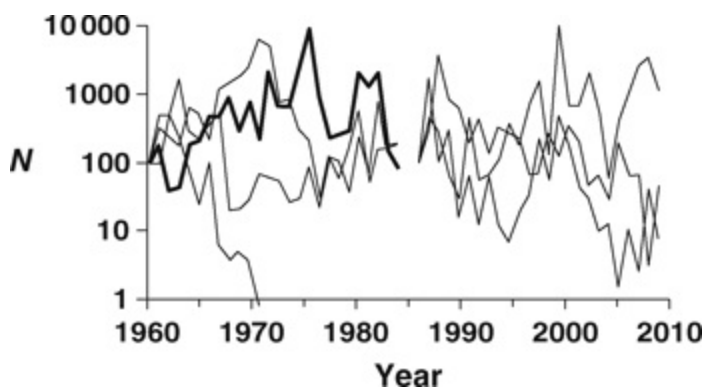
$$r = \ln\left(\frac{175}{90}\right) = 0.665$$

In year 3, the numbers were 40, so for the year 2 to year 3 transition  $r$  is as follows:

$$r = \ln\left(\frac{40}{175}\right) = -1.476$$

A stationary population has  $r = 0$ , while positive  $r$  indicates a growing population and negative  $r$  a declining population. The mean  $r$  for this Bay checkerspot butterfly population was 0.002 (essentially stationary) and its variance 1.456.

The stochastic projection for this population, obtained using  $r$ -model software, is shown in the figure below. Projections were prepared both to compare with the actual population trajectory for 1960–86 (bold), and to project forwards for an additional 25 years. Only three replicates are shown (to avoid cluttering the figure).



Even simpler methods may be used in predicting the future fate of populations when data are scarce. Some rely on surveying the opinions of experts (but non-quantitative methods have a poor record). For example, Ralls & Starfield (1995) used methods from business management to evaluate strategies for managing endangered Hawaiian monk seals, where males occasionally mob and kill females. The IUCN categorization system using criteria A–D also represents a simple means of inferring extinction risk (Chapter 1).

## Genetics and PVA

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PVAs reveal that inbreeding depression usually increases the long-term risk of extinction in threatened species

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Currently, inbreeding depression is the only genetic threat that has been incorporated into PVAs. For this incorporation, we need to know:

- susceptibility of the species to inbreeding depression
- fitness components affected by inbreeding
- whether the relationship between  $F$  and inbreeding depression is linear or curvilinear
- the extent of purging, which is strongly dependent on proportions of alleles that are overdominant, deleterious (sub-lethal) or lethal
- the extent of isolation among fragments, i.e. the gene flow rates, as these affect the inbreeding coefficient
- the breeding system (outbreeding versus selfing, monogamous versus polygamous versus hermaphrodite, etc.), as this affects  $F$
- population size and sex-ratio of breeders.

Where information is not available for the specific species, information from other, related species may be used, especially when based upon meta-analyses. For example, if susceptibility to inbreeding is not known, the median lethal equivalents (3.14) for captive mammals for juvenile mortality from Ralls *et al.* (1988) has often been used. However, 12 lethal equivalents is a more appropriate default value for wild populations (O'Grady *et al.* 2006). Perhaps the best default for the extent of purging is to assume that 50% of deleterious alleles are lethal and the rest are alleles of small effect, as occurs in fruit flies (Simmons & Crow 1977).

As discussed in Chapters 2 and 13, PVAs have revealed that inbreeding depression substantially increases extinction risk for a broad range of species (O'Grady *et al.* 2006).

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PVAs typically do not encompass the full genetic impacts on population viability

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Most PVAs ignore genetic issues, and are likely to seriously underestimate extinction risk (Chapters 2, 13 and 17). Even when genetic factors are included (typically using VORTEX), inbreeding depression is normally only imposed on juvenile survival, even though inbreeding depression affects reproduction, survival, mating ability, parental care and longevity (Chapter 13). However, it is now possible to incorporate inbreeding depression for other components of fitness, using recent versions of VORTEX (O'Grady *et al.* 2006).

Further, PVA packages typically assume Poisson variation in family sizes and thereby underestimate the reduction in  $N_e$ , as populations usually have



higher variances (Table 11.2). To date, evolutionary potential and any impacts of new mutations are ignored in all PVAs. Estimates of the population size required to retain evolutionary potential (that ignore all other demographic and genetic factors) are of a similar magnitude to those that include all deterministic and stochastic factors, apart from evolutionary potential (Table 22.2). Consequently, PVAs will typically underestimate genetic impacts on extinction risk.

**Table 22.2** *Sizes required for long-term viability of populations to cope with different threats. Variation refers to the propensity for population sizes to fluctuate*

	$N_e$	$N$	Reference
<b>Theory</b>			
1. Genetic diversity	500–5000	5000–50 000	1
2. Mutational accumulation	1000	10 000	1
3. Demographic stochasticity		10s–100	1
4. Environmental stochasticity		1000+	1
5. Catastrophes		1000+	1
<b>Empirical data</b>			
PVA for 100 vertebrate species: 99% probability of persistence for 40 generations		>6000	2
Maintain 95% of initial fitness in plant populations		2500	3
Primates in Sunda Islands		>16 000	4
99% probability of persistence for 40 generations for 1198 species, from <i>r</i> -models		1100	5
PVA for 212 species: 99% probability of persistence for 40 generations		4169	6
Mammals (95 <sup>a</sup> )		3876	6
Birds (48)		3742	6
Amphibians and reptiles (31)		5409	6

	$N_e$	$N$	Reference
Fish (8)		1 239 727	6
Insects (5)		10 841	6
Marine invertebrates (3)		3611	6
Plants (22)		4824	6

<sup>a</sup> Number of species.

*References:* 1, Nunney & Campbell (1993); 2, Reed *et al.* (2003c); 3, Reed (2005); 4, Harcourt *et al.* (2002); 5, Brook *et al.* (2006); 6, Traill *et al.* (2007).

## Insights into the causes of extinction from PVA

PVA has been used to investigate the relative contributions of different factors to extinction risk, and thus to improve our understanding of the causes of extinction.

The impacts of demographic, environmental and genetic stochasticity and catastrophes in reducing population growth and increasing extinction risk have been demonstrated in PVA simulations. For example, we saw that inclusion, versus exclusion, of inbreeding depression increased extinction risk in models for a broad range of species (Fig. 2.4 and Box 17.2).

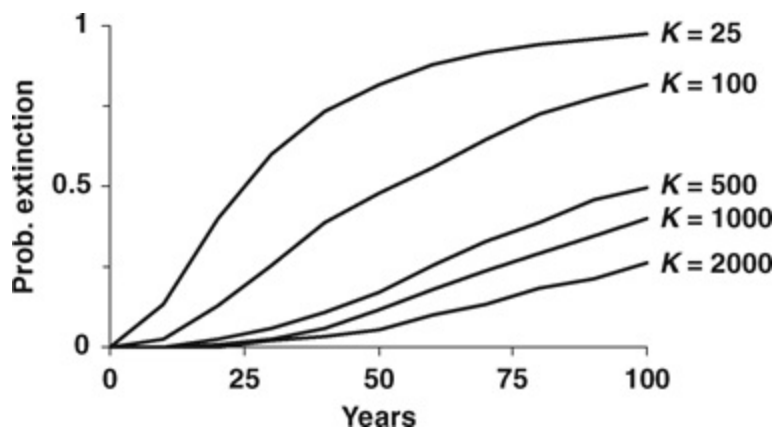
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Demographic, environmental and genetic stochasticity reduce population growth and increase extinction risk

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PVAs predict that extinction probabilities for Eld's deer are highest in the smallest population and lowest in the largest ones, and increase with time (Fig. 22.5). Similar relationships are evident in real populations, including birds, bighorn sheep (Fig. 2.3), small mammals, Lepidoptera and several species of plants (Matthies *et al.* 2004). Further, there are positive relationships between island area (an indicator of population size) and number of species (Terborgh & Winter 1980; Diamond 1984) and between extinctions and habitat area in both National Parks and mountain tops in North America (Chapter 2).

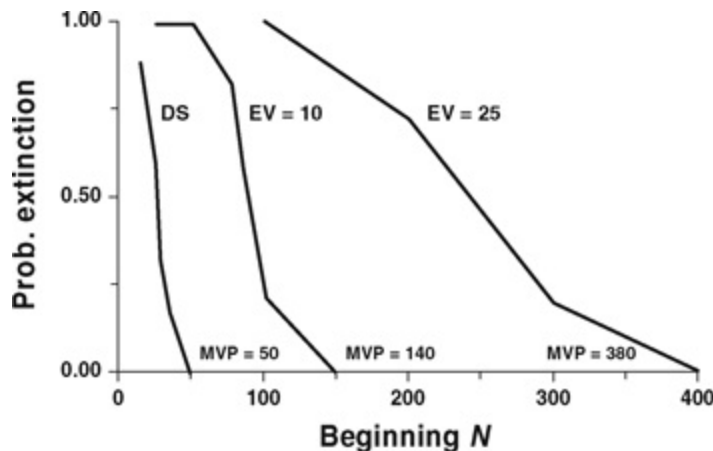
Small populations have higher extinction risks than large populations of the same species



**Fig. 22.5** Relationship between extinction risk and population size. Cumulative extinction risks for endangered Eld's deer (from Myanmar) over time in five populations with different carrying capacities ( $K$ ) (based on data from Wemmer *et al.* 2000). *Extinction risk increases with time and is greater in smaller than in larger populations.*

Demographic stochasticity increases extinction risk, but it has little impact in populations with sizes of greater than 50–100

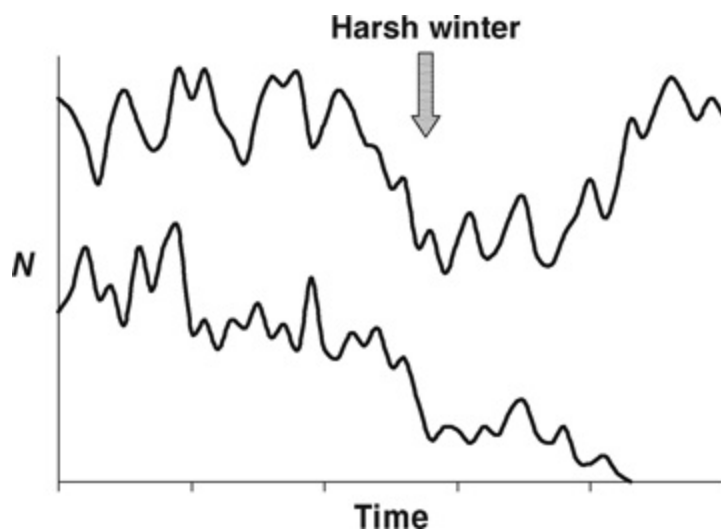
The impact of demographic stochasticity is inversely proportional to population size. For example, the variation in sex-ratio for a population with a 1 : 1 sex-ratio is  $0.5/\sqrt{N}$ . Thus, impacts are minor when the population size ( $N$ ) reaches 100. Extinction risk due to demographic stochasticity in the plant *Astrocaryum mexicanum* over 100 years is very high in populations of 10, but negligible above population sizes of 50 (Fig. 22.6).



**Fig. 22.6** Extinction probabilities plotted against initial population sizes for the plant *Astrocaryum mexicanum* for models with demographic stochasticity (DS) alone, or two levels of environmental variation (EV = 10 and EV = 25). Population sizes required to limit extinction probabilities to 5% over 100 years (MVP) are given for each case. ‘Extinction’ was defined as 10 or fewer individuals (quasi-extinction) (after Menges 1992).

Even large populations are at risk of extinction due to environmental

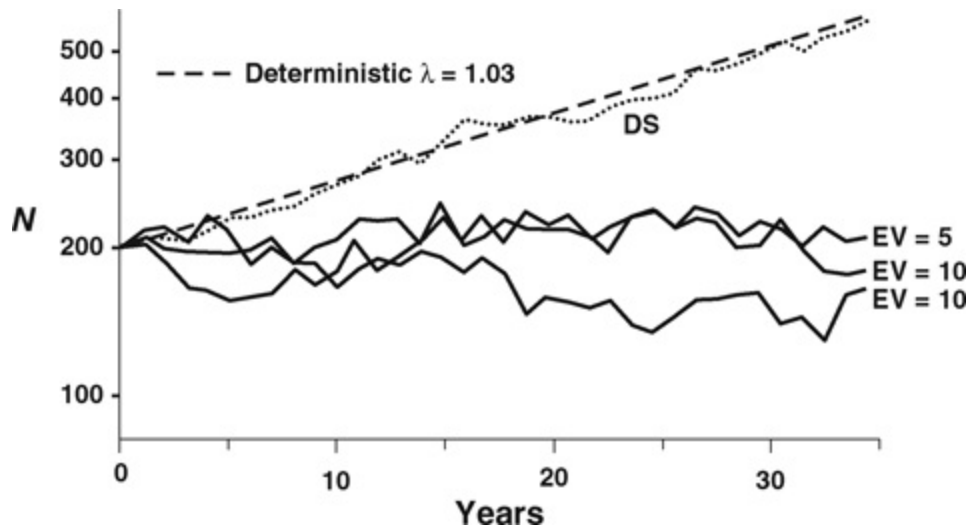
Environmental stochasticity increases extinction risk at much larger population sizes than demographic stochasticity. [Figure 22.7](#) shows that similar fluctuations in population size may result in a large population persisting, but drive a smaller population to extinction. For example, populations have to be above 140 before the extinction risks from low levels of environmental stochasticity over 100 years is restricted  $\leq 5\%$  ([Fig. 22.6](#)).



**Fig. 22.7** Impact of environmental stochasticity on large and small hypothetical populations. While the fluctuations are similar in both, the large population persists but the small population becomes extinct.

The comparative impacts of demographic and environmental stochasticity on population size are illustrated in [Fig. 22.8](#). In populations beginning at size 200, environmental stochasticity markedly depresses population growth and increases extinction risk, compared to the deterministic case, while demographic stochasticity has no obvious effect. Further, extinction risks increase as the level of environmental variation increases. For example, in *Astrocaryum mexicanum*, the population size required to limit extinction probabilities to  $<5\%$  over 100 years was 140 with low environmental

variation ( $EV = 10$ ) and 380 with high environmental variation ( $EV = 25$ ) (Fig. 22.6). Experimental data on water fleas confirmed that higher environmental variation results in higher extinction risk (Drake & Lodge 2004).



**Fig. 22.8** Impact of environmental stochasticity (EV) and demographic stochasticity (DS) on population growth in the plant *Astrocaryum mexicanum* compared to deterministic growth (after Menges 1992). With an initial population size of 200, environmental stochasticity depresses population growth, but demographic stochasticity does not.  $\lambda$  = annual population growth rate.

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Catastrophes increase extinction risk, with their impact depending upon their frequency and severity

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Catastrophes, due to hurricanes, severe winters, fires and disease epidemics often have substantial effects on extinction risk in smaller populations. For example, a PVA on the Puerto Rican parrot (Box 22.1) identified

catastrophes in the form of hurricanes as a major risk. Shortly after the PVA was completed, a hurricane hit the island and reduced the bird's population. The black-footed ferret was sent to extinction in the wild in 1987, primarily through a catastrophic canine distemper outbreak (Clark [1994](#)).

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### **Box 22.1 Population viability analysis for the Puerto Rican parrot**

The endangered Puerto Rican parrot declined to 13–14 birds in the wild because of deforestation, poaching for the pet trade, predation and hurricanes. A captive population was established by removing eggs from wild nests. A PVA was conducted when the wild population had about 40 birds (Lacy *et al.* [1990](#)). It predicted a probability of extinction of over 30% over 100 years, primarily due to catastrophes from hurricanes. Consequently, stockpiling of food for the parrots at the remote breeding centre was recommended, so that birds could be fed if a hurricane cut access roads. It also recommended that a population be established off the island. The long-range plan called for the establishment of five independent wild parrot populations in Puerto Rico.

The recommendations were prescient! Soon after, the population suffered a serious setback from a hurricane that devastated the forest habitat (Seal [1991](#)). The stockpiled food allowed captive birds to be fed whilst the Centre was isolated, and prevented their extinction. Careful genetic management was recommended for this small population as it exhibits inbreeding depression (Brock & White [1992](#)). The management plan has subsequently been criticized on the basis of the undesirability of fragmenting the population and on the disease risk posed by returning captive-bred birds to the wild (Wilson *et al.* [1994](#)).



Puerto Rican parrot

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Immigration may ‘rescue’ populations and thus allow small populations to persist. Rescue can be due to either demographic or genetic factors, but often involves both

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Populations that are too small to persist on their own may be ‘rescued’ by immigration from other small populations that are experiencing relatively independent fluctuations in population size. For example, a population of acorn woodpeckers in the American Southwest, with a carrying capacity of 52, was predicted to have a median persistence time of only 16 years (and a maximum of 48 years). However, the real population had persisted for more than 70 years (Stacey & Taper [1992](#)). Addition of five immigrants per generation into the model dropped the probability of extinction over 1000 years to zero. The real population was subsequently found to receive about 10



immigrants per year. In general, the ‘rescue effect’ of immigration will usually be even greater when inbreeding depression is included in models (Richards [2000](#); [Box 17.2](#)).

## Recovering threatened populations

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Population viability analysis is widely used as a management tool to compare options to recover species

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The CBSG and other agencies have conducted many PVAs to compare alternative recovery options, including legal restraints on exploitation, predator removal, habitat improvement, reserving habitat, captive breeding, etc. Typically, these commence with sensitivity analyses, followed by detailed PVAs that compare a range of specific management options.

## Sensitivity analyses

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Sensitivity analyses involve determining the relative impact of variation among input parameters on extinction risk

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Sensitivity analyses involve varying input parameters by increments in either direction around their mean and evaluating the effects on extinction risk or population trajectories (Wisdom & Mills [1997](#); Mills & Lindberg

2000). In this way, the input parameters whose values most influence the output can be identified. For example, is the outcome most sensitive to varying juvenile survival, or adult reproductive rate, or predation level? First-year survival was the parameter whose variation had most impact on population growth rate in the greater prairie chicken, leading to the recommendation that management should focus on finding ways to improve nest success, brood survival and post-brood survival to one year of age (Wisdom & Mills 1997).

Sensitivity analyses have altered our perceptions about the most important factors threatening cheetahs and loggerhead sea turtles. Predation on cheetah cubs by lions and hyaenas had been viewed as the major threat to the cheetah's viability (Caro & Laurenson 1994). However, a sensitivity analysis revealed that cheetah population numbers were much more sensitive to changes in adult than juvenile survivorship (Crooks *et al.* 1998). In loggerhead turtles, management had focused on increasing hatchling survival to reverse population decline, but a sensitivity analysis showed it was more efficient to reduce adult mortality from capture in shrimp nets (see Mills & Lindberg 2000).

## **Using PVA to evaluate management options: case studies**

The following represent a selection of case studies chosen to represent a diversity of PVA analyses where threats and management options differ.

### **Black-footed ferrets**

The black-footed ferret once occupied an area of 40 million hectares, but its numbers were drastically reduced by control of its prairie dog prey on agricultural lands. They were thought to be extinct by the late 1970s, but in 1981, a population of about 80 individuals was discovered in Wyoming (Seal *et al.* 1989; Clark 1994). Initial recovery efforts concentrated on maximizing the number of wild ferrets, and maximizing the number of prairie dog prey. An initial risk analysis identified stochastic factors (genetic, environmental

and demographic stochasticity plus catastrophes) as potential problems. Several studies recommended captive breeding and reintroductions. This was not implemented until after a subsequent catastrophic decline of the ferrets in 1985, due to bubonic plague among the prairie dogs and an epidemic of canine distemper in the ferrets. The initial program was bedevilled by bureaucratic and political problems. All remaining animals were taken into captivity by 1987, with 18 individuals surviving to initiate the captive population.



Black-footed ferret

A PVA in 1989 indicated that about 120 animals were required for a wild population to show greater than 95% probability of persisting for 100 years. Multiple independent populations were considered to provide the best insurance against overall loss of the species from catastrophes (e.g. from disease in the ferrets, or their prey). There were additional serious genetic concerns about the population that this model did not include (i.e. loss of genetic diversity and inbreeding depression). The goals of the US Fish and Wildlife Service black-footed ferret recovery plan in 1987 were to increase the captive population to an effective size of 200 breeding adults by 1991, and to establish wild populations of 1500 adults in 10 or more populations by 2010 (a metapopulation).

The captive population has now increased in size to about 300 adults and >11 wild populations have been established, but only two of the reintroduced populations have been particularly successful to date ([Chapter 20](#)).

## **South African leopards**

Leopards in South Africa inhabit ten core areas (with populations ranging in

size from 20 to 2000 animals) (Daly *et al.* 2005). About 280 leopards are removed from the metapopulation each year via legal and illegal hunting, removal of problem animals, and emigration. The controlled legal hunt has a maximum annual harvest of 75 leopards. Additionally, about 30 leopards move into the population each year from neighbouring countries. Under these conditions PVA models project that, while some of the smaller populations have a moderate probability of going extinct, the metapopulation is likely to persist over the next 100 years with relatively little loss in numbers or genetic diversity.



Leopard

In 2004, at the request of hunters and farmers (leopards kill some livestock), the CITES authorities doubled the number of legal harvests to 150. However, this was based on minimal ecological information and few data on the rate of illegal harvests. PVA modelling indicates that no more than an additional 69 leopards (total 75 + 69) and possibly fewer, can be removed from the South African metapopulation. An increased harvest can only be sustained in four of the populations and in the smaller populations even a slight increase in harvest increases the possibility of local extinction. The modelling results and recommendations from the PVA workshop were presented to CITES officials and the government biodiversity directorate and, in the interim, no additional leopard hunting permits have been issued. The **population and habitat viability assessment** (PHVA) report is being considered as the primary input into this decision-making process.

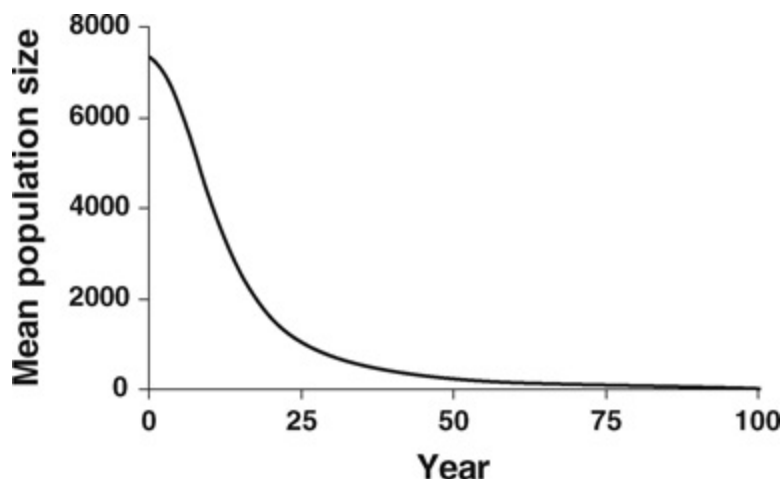
## Orangutans

Orangutan populations in both Borneo and Sumatra are seriously threatened as widespread logging converts forests into plantations (Singleton *et al.* 2004). While some protected areas exist, neglect since 1998 led to large-scale illegal deforestation, regardless of the official status of the land and to steady decline in orangutan populations.



Orangutan

In Sumatra, orangutans currently inhabit 13 different areas, ranging in population size from about 40 to 2500, with a total number of about 7500. A PHVA held in 2004 showed that current rates of logging, habitat loss and other factors will cause Sumatran orangutan populations to decline rapidly toward extinction (Fig. 22.9). They are expected to decline by 50% in about a decade, by 97% in 50 years, and to eventually disappear unless continued habitat loss is stopped (unlikely).



**Fig. 22.9** Mean population size of all surviving orangutan populations in all 13 habitat units in Sumatra over the next 100 years under current rates of logging.

Borneo has ~50 habitat units containing orangutans, with a total of ~50 000 animals of three sub-species. Modelling shows that, with an increase in logging, even populations that are currently very large could be driven to extinction within the next 50 years. The priority recommendations focused on educating and influencing the government to support conservation programs and reduce logging.

## Whooping cranes

By the late 1930s, the last remaining refuge of the endangered whooping cranes was in and around the Arkansas National Wildlife Refuge on the Gulf of Mexico coast in the USA (Mirande *et al.* 1991). These birds migrated during the summer to northern Alberta and the Northwest Territories in Canada. In 1941 the population underwent a bottleneck that reduced the population to only 15 birds (six or eight breeders). However, the population has recovered rapidly and, as of the end of 2006, there were 518 birds.



Whooping crane

A captive population was established in 1967. In 1993, a non-migratory flock was established in Florida. To explore what might be involved in establishing these flocks, a PVA was conducted in 1991 to determine whether the captive population (at that time about 70 birds) could sustain the harvest rate needed to ensure a successful reintroduction in Florida. The model output indicated that, with improved management, the captive population could sustain release rates of 10 to 20 birds per year, but only if the captive population was allowed to grow for three more years before regular harvest. By 1993–94 the captive population had grown to 89 birds, and the reintroduction program was initiated in February 1993 with the release of 14 birds. Between 19 and 48 cranes have been released in each subsequent year.

Loss of genetic diversity and inbreeding depression are also a concern due to the bottleneck (Glenn *et al.* 1999; Jones *et al.* 2002). To address this issue, the birds were genotyped for 11 microsatellite loci and are being managed based on revised pedigrees and similarities based on sharing of microsatellite alleles.

## **Chinook salmon**

Chinook salmon in Oregon, USA have declined dramatically since early last century, primarily due to habitat degradation associated with siltation from road building and forestry. Ratner *et al.* (1997) conducted a PVA on the spring chinook population in the South Umpqua River which averaged fewer than 300 spawners per year. Projected extinction risks over 100 and 200 years were very low, assuming no further habitat degradation. However, this conclusion was highly sensitive to uncertainty about density dependence. The projected extinction risk was 100% assuming habitat degradation continuing at a rate similar to that in the past. Several PVAs have been run on the Snake River populations of this species (Zabel *et al.* 2006). The prospects for these populations depend critically upon the climatic conditions and continued global warming leads to a pessimistic outlook.



Chinook salmon

## **Furbish's lousewort**

About 5000 individuals of this endangered herbaceous perennial live in 28 colonies along a 230-km stretch of a single river in Maine and New Brunswick in northeastern North America. It is limited to periodically disturbed, north-facing riverbanks. The lousewort is an early successional hemiparasite that cannot invade disturbed riverbanks for at least three years, but is later crowded out by taller competitors, leading to regular rounds of colonization and population extinction (the species exists as a metapopulation). A PVA predicted that individual populations had 87% probabilities of extinction within 100 years, and that the survival of the species is critically dependent on the balance between colonization and extinction (Menges [1990](#)). As extinctions currently exceed colonization, the long-term viability of this species is tenuous. Further, the long-term ability of the population to adapt is questionable as all surveyed populations of the species lack genetic diversity at 22 allozyme loci (Waller *et al.* [1987](#)).





Furbish's lousewort

## **Matchstick banksia**

The vulnerable matchstick banksia is a large shrub native to the southwest of Western Australia. There are about 340 plants confined to seven populations over a range of 60 km. Recruitment occurs mainly after fires when seed stored in the canopy is released and mature plants are killed. The dynamics of this species are mainly controlled by variation in rainfall, and the frequencies of controlled fires and wildfires. A PVA incorporating demographic, environmental and genetic factors revealed that the options to maximize population size and to minimize the risk of extinction are different (Burgman & Lamont [1992](#)). Mean size is increased by a moderate frequency of controlled fires at 11–25-year intervals, but this regime results in an extinction risk of about 50% over 50 years, as intensive fires can destroy populations. The risk of extinction is minimized when the frequency of fires is kept as low as possible, but this leads to a substantial decline in population size. If rainfall declines over the next 50 years as a consequence of climate change, the species has a low probability of persistence, even in the absence of controlled fires. The only way to ensure a reasonable chance of persistence of the species is to intervene by watering seedlings whenever there is severe drought following a fire. Inbreeding depression has limited impact on this species over 50 years as it is long-lived (mean generation length of 23 years).

## **How useful are the predictions of PVA?**

### **The PVA process may be more important to conservation than the PVA output**

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PVA often has its greatest value as a heuristic tool to assist planning for the recovery of threatened species, to allow iterative planning, to determine sensitivities and to compare recovery options, rather than in

providing accurate predictions of extinction risk
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As insufficient life-history data exist for most threatened species, full population viability analyses may not be possible, or they may have low reliability. However, the most important contributions of risk assessments using PVA do not necessarily come from the quantitative assessments of extinction risk themselves (Burgman [2006](#)). Rather, the process of conducting a PVA involves:

- summarizing information about the life history of the species
- identifying all the threatening processes impacting upon it
- assessing their likely importance (sensitivity analyses)
- identifying potential recovery strategies and evaluating their relative impacts
- identifying deficiencies in knowledge about the species, and formulating research proposals to remedy them.

Thus, considerable benefits may be gained by the PVA process, even if the quantitative predictions are not particularly accurate. PVA provides a transparent planning process that has internal consistency and should indicate uncertainties in predictions. Further, the recovery process can be operated in an adaptive manner. PVA projections can be updated as more information is gathered and management practices modulated in light of PVA predictions. The main alternative to PVA analyses is human judgement and this is notoriously inaccurate in many diverse disciplines involving complex systems, including finance, medicine, climate prediction and biology (Zeckhauser & Viscusi [1990](#)).

A PVA is best conducted in a workshop environment involving all the experts on a given threatened species, as is typically used by the CBSG in their population and habitat viability assessment workshops (PHVA). Deficiencies in knowledge about the species are identified, often leading to

the formulation of research proposals to improve our knowledge. Unpublished results and expert knowledge may become available for inclusion in the PVA. Groups with strongly divergent views can be ‘encouraged’ to cooperate at workshops. Further, the PVA process increases the probability that funds for recovery programs or research will be made available.

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PVA typically involves meetings of all the experts on a species so that deficiencies in knowledge are identified and cooperation in recovery efforts is encouraged

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## **How accurate are the predictions of PVA?**

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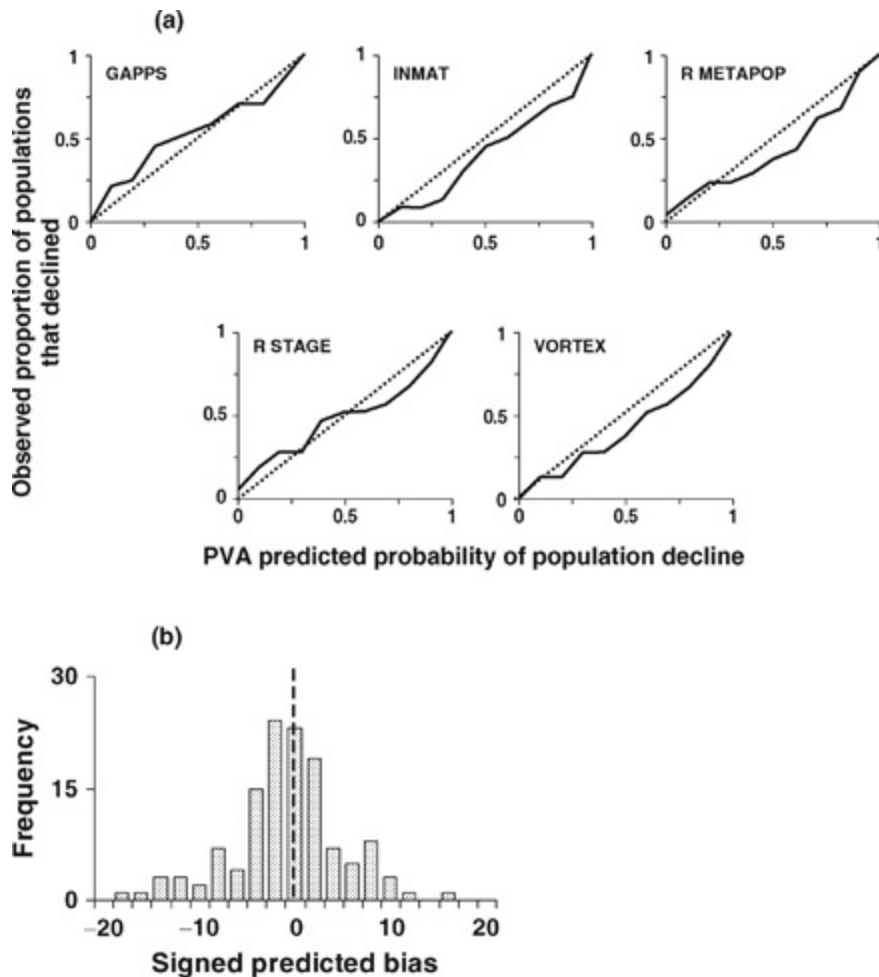
PVA provides unbiased average predictions of the future fate for well-studied populations, but predictions for individual populations are highly variable

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There was wide-ranging scepticism about the accuracy of PVA predictions based on questions about insufficient data, ignoring threatening factors and adequacy of models (see Caughley & Gunn 1996; Ludwig 1999). However, an empirical evaluation indicated that it predicted population fate without bias for 21 well-studied taxa (Fig. 22.10). Further, predictions from different software packages were highly correlated with each other and with predictions from simple stochastic *r*-models. A simulation study of the

predictive accuracy of PVA reached a similar conclusion, with predictions being reasonable when based on 10 or more years of data (McCarthy *et al.* 2003). PVA predictions should be compared with daily weather forecasting, long-term weather forecasting and economic projections which also contain uncertainty, but are valuable for planning. These are all readily accepted by society, which also recognizes their less than perfect predictive abilities.



**Fig. 22.10** Predictive accuracy of five PVA software packages (Brook *et al.* 2000). (a) Plot of predicted versus observed risks of population size decline in retrospective studies on 21 well-studied species. Data from the first half of each study were used to predict numbers in the second half. A perfect fit to reality lies on the 45° line. (b) Differences between projected and observed population size. *PVA predicted without significant bias.*

Evaluations of the fate of metapopulations yielded relatively poor

predictions, but this may be accounted for by lack of reliable information on dispersal rates (Lindenmayer & Lacy 2002; Ball *et al.* 2003; Box 14.2). In general, predictions for metapopulations will be less precise, as more parameters need to be estimated and included in the computer projections.

PVA therefore appears to be suitable for the conservation purposes for which it is being used. To be reliable, PVA models must adequately reflect important aspects of species biology. For example, the poorest projection in the Brook *et al.* (2000) study was for the Soay sheep using INMAT software, where the package could not realistically model the over-compensatory density dependent population cycle shown by the sheep (Chapman *et al.* 2001). Similarly, predictions were good when the correct form of density dependence was used in models for laboratory populations of water flea, but severely biased when it was not included (Drake 2005). Whilst information on density dependence is often lacking for threatened species, analyses of long-term data sets indicate that the great majority of well-studied species of mammals, birds, fish, reptiles/amphibians, insects and plants do show density dependence (Sibly *et al.* 2005; Brook & Bradshaw 2006). Consequently, where information on density dependence is inadequate for a particular species, it is advisable to include it in PVA models by using default parameters taken from closely related taxa or species with similar life histories.

## **Lessons learned**

Shaffer *et al.* (2000) provided an excellent overview of the lessons learned from PVA, but not acted upon: (a) the major limitation of PVA is lack of detailed population data, (b) there is a consensus that populations of fewer than a few thousand individuals are of questionable viability, but recovery goals for many threatened populations are less than this, and (c) there is no agreed and workable definition of population viability. We elaborate upon these issues below.

## **Limitations of PVA**

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A major limitation of PVA is that there is usually limited information available on the biology of threatened species

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For most threatened species, there is little information on numbers, even less on age-specific mortality and fecundity, and very rarely reliable estimates of variances for parameters. Often the primary cause(s) of species decline are unknown (Caughley & Gunn 1996). An accurate PVA requires sound data. Where only some information is lacking, information from other species may be used, e.g. information on the frequency and severity of catastrophes, where these are similar across a broad range of vertebrate taxa (Reed *et al.* 2003b). In some cases, simple stochastic *r*-models can be used to project the population fate, but these are likely to underestimate extinction risk as they do not include impacts of genetic factors (Chapter 2). Further, they do not usually lend themselves to evaluation of management options, as they do not permit sensitivity analyses.

We are surprisingly depauperate in our basic knowledge of the biology and ecology of endangered species. Consequently, long-term field studies need to be developed (Shaffer *et al.* 2000).

Some PVAs assume that environmental conditions will remain unchanged into the future, but this is doubtful in a scenario of global climate change. For example, the frequency of weather-related catastrophes has increased six-fold since the 1950s (Schiermeier 2006). PVAs can (and should) evaluate the likely impacts of climate change, as was done for the Lord Howe Island woodhen (Brook *et al.* 1997a), and for the Arizona cliff rose (Maschinski *et al.* 2006).

## What is a viable population?

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There is no objective definition of viable population

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A viable population is loosely defined as one with a high probability of surviving for a long time. How high? How long? These are undefined quantities. However, 99% or 95% survival probability is most frequently used for the former, and time spans of 100 to 1000 years are normally considered for the latter (Shaffer [1981](#); Soulé [1987](#)). Shaffer ([1981](#)) defined a viable population as having a 99% chance of remaining extant for 1000 years. IUCN ([2007](#)) defines lower risk populations as having greater than a 90% probability of surviving for 100 years.

These definitions all use years as their time frames. However, extinction risk scales better to generations than to years (Frankham & Brook [2004](#); O'Grady *et al.* 2008). Consequently, viable populations would more appropriately be defined in terms of persistence for a defined number of generations, say 99% persistence for 40 generations, as used by Reed *et al.* ([2003b](#)). Further, it is advisable when obtaining default parameters from other species to analyse data on a per-generation scale, as many more species can then be included on an equal footing. This was done by Reed *et al.* ([2003b](#)) for information on the frequency and severity of catastrophes.

## Minimum viable population sizes (MVP)

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There is a consensus that the size required for a population to be viable in the long term is at least thousands to tens of thousands of individuals

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Estimates of the minimum size of population required, based on a variety of theoretical arguments and on PVAs, all indicate that numbers in the thousands to tens of thousands are required for populations to be viable in the long term (Belovsky 1987; Soulé 1987; Table 22.2). Thomas (1990) argued empirically that 10 is far too small, 100 is usually inadequate, 1000 is adequate for species of normal variability in population sizes, while 10 000 should permit medium- to long-term persistence of birds and mammals that exhibit strong fluctuations in population size.

It is widely assumed that the required size is not universal, but depends on details of the biology and environment of the species. However, this assumption appears to be an artefact of defining it for a fixed number of years, rather than generations. The MVPs predicted for 99% persistence for 40 generations for vertebrates did not differ significantly across major taxa, or between different climatic zones in a study of 100 species with varying life histories and geographic ranges (Reed *et al.* 2003c). However, there was an effect of study duration on MVP, as longer data sets captured larger variances and led to larger MVPs. The meta-analysis of MVP estimates by Traill *et al.* (2007) provides the best practical guidelines on MVP, as it is based on 212 species and is standardized to include all threatening processes, apart from loss of evolutionary potential. There is a suspicion that MVP may differ among major taxa, but this is uncertain due to the limited number of studies in some taxa (Table 22.2).

## **Minimum habitat area**

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Minimum habitat areas for long-term population persistence can be determined from MVP and the habitat required per individual for the species



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In practice, the desired information for species conservation in nature may be the minimum habitat area of reserves and national parks required for a high probability of long-term persistence. Minimum habitat area can be estimated from minimum viable population sizes and habitat requirements for the species, as illustrated in Example 22.2 for golden lion tamarins. A minimum area of 24 km<sup>2</sup> is required for the tamarins to have a 90% chance of persistence for 100 years.

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### **Example 22.2 Minimum habitat area for golden lion tamarins**

The minimum viable population size required for a 90% probability of persistence for 100 years in golden lion tamarins has been estimated as 175 tamarins, based on PVA analyses (J. J. O'Grady unpublished data). The minimum habitat area is estimated by multiplying the above number by the habitat requirement per tamarin (one tamarin per 13.9 hectares: Ballou *et al.* 1998). Thus, the minimum habitat area (MHA) is

$$\begin{aligned}\text{MHA} &= \text{MVP} \times \text{habitat requirement} = 175 \times 13.9 \text{ ha} = 2433 \text{ ha} \\ &= 24.3 \text{ km}^2\end{aligned}$$

The two existing reserves for golden lion tamarins encompass 79 km<sup>2</sup>, about 50% of which is suitable forested habitat. (The actual goal is a 98% probability of persistence for 100 years, and a larger area is required for this: Ballou *et al.* 1998.)

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From the above it is clear that populations must have sizes at least in the order of a few thousand to be viable in the long term. Both the population

size at the time species are listed as threatened, and the recovery targets under the USA Endangered Species Act are typically too small (Shaffer *et al.* 2000; Reed *et al.* 2003c). The median size at listing is about 1000 individuals for animals and 100 for plants. Further, the median population size for a taxon to be considered recovered is about 1550.

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Population sizes used to list and de-list threatened species are usually less than those recommended above

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A worrying implication of these numbers is that even the largest reserves are too small to maintain adequate population sizes for long-term survival of large herbivores and especially large carnivores (Shaffer 1987). However, assisted migration among several reserves may allow such species to be viable in the long term.

While major advances have been made in the science underlying conservation of threatened species, it is often not matched by appropriate action.

## Summary

1. Wild populations face threats from both deterministic factors (habitat loss, over-exploitation, pollution and introduced species), and stochastic threats associated with small population size.
2. Small populations face threats due to demographic stochasticity, environmental stochasticity, catastrophes and genetic stochasticity. Genetic stochasticity encompasses inbreeding depression, loss of genetic variation, population divergence and the accumulation of

new deleterious mutations.

3. Extinction risk can be predicted using population viability analysis. Typically values of reproductive and survival parameters, population size, carrying capacity, along with information on environment and its variation, inbreeding depression, habitat quality and loss, etc., are input to computer packages and stochastic projections made.
4. Population viability analysis is widely used as a management tool to compare different options to recover a species.
5. The size required for a population to be viable in the long term is several thousands, based on PVAs.
6. Recovery of small threatened populations involves reversing both the original cause of decline, and addressing stochastic threats.

### **Further reading**

Beissinger & McCullough (2000) *Population Viability Analysis*. Proceedings of a conference on PVA. See especially Mills & Lindberg, Ralls *et al.* and Shaffer *et al.*

Burgman (2006) Brief review on role of PVA in conservation biology, especially in the face of limited data.

Mills (2007) *Conservation of Wildlife Populations*. Textbook covering the topics in this chapter.

Morris & Doak (2002) *Quantitative Conservation Biology*. Textbook on PVA.

Reed *et al.* (2003c) Uses PVA to determine minimum viable population sizes for 100 vertebrates species and evaluated variables affecting MVP.

Traill *et al.* (2007) A meta-analysis encompassing the MVP estimates obtained in the last 30 years, plus analyses on factors affecting their magnitudes.

Wilcove (1994) *The Condor's Shadow*. Enjoyable and informative popular book on the decline and recovery of wildlife in America.

### **Software**

VORTEX: Individual-based population viability analysis package that can encompass effects of inbreeding depression and fragmented populations (Lacy *et al.* 2005). [www.vortex9.org/vortex.html](http://www.vortex9.org/vortex.html)

RAMAS: A family of commercial packages for matrix-based PVAs, including RAMAS GIS (incorporating RAMAS Metapop) and RAMAS Landscape, both able to model meta-populations. Particularly suitable for highly fecund animals and plants. [www.ramas.com/](http://www.ramas.com/)

## Problems

22.1 Computing  $r$  and its variance. For a second population of Bay checkerspot butterflies, determine for each generation transition  $\lambda$  and  $r$ , and so calculate the mean  $r$  and the variance of  $r$  (data from Foley 1994).

Year	N	Year	N	Year	N
1960	70	1970	820	1980	125
1961	350	1971	235	1981	316
1962	750	1972	1149	1982	109
1963	750	1973	370	1983	122
1964	1400	1974	177	1984	31
1965	2000	1975	317	1985	48
1966	1750	1976	1001	1986	18
1967	900	1977	190		
1968	576	1978	341		
1969	871	1979	135		

22.2 Management for recovery of endangered species. What action would you suggest to recover the northern hairy-nosed wombat (see Box 11.3)?

22.3 Management for recovery of endangered species. What action would you suggest to recover a species of endangered *Partula* snail from Tahiti that is being predated by an introduced carnivorous snail?

22.4 Management for recovery of endangered species. What action

would you take to recover the Sumatran tiger? All tigers are threatened by habitat loss and harvesting for the Asian medicinal market. The Sumatran tiger has six isolated populations in reserves, plus a few other populations.

## **Practical exercises: Population viability analyses**

Population viability analyses can be carried out on any threatened species where you can find adequate data. The following are suggestions.

(Note: Input files for VORTEX have changed slightly with versions.)

1. Stochastic  $r$ -model. For the population of Bay checkerspot butterflies described in Problem 22.1, use the mean  $r$  and variance of  $r$  to project the population forward for 50 years using  $r$ -model software. Run 50 replicates to evaluate the variance in outcomes. Begin runs with the average population size. Further options that can be tried are: (a) vary the starting population size and examine the extinction probability, (b) examine the fate of populations with positive, zero and negative  $r$  values, and (c) evaluate the effects of increasing and decreasing the variance of  $r$ .
2. Comparing management options using PVA. For the Lord Howe Island woodhen, use VORTEX simulations to compare the following options for recovery of the population when it was at a size of 20: (a) no action, (b) pig control alone (increase the carrying capacity from 20 to 200), (c) captive breeding program with three pairs of founders (supplement the population with 30 individuals in each of years, 1, 2 and 3), and (d) captive breeding plus pig control. Compare the probabilities of persistence with these scenarios. Information for input files is given in Brook *et al.* (1997b).

## **Take home messages from this book**

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1. The biological diversity of the planet is being rapidly depleted due to direct and indirect consequences of human activities (habitat destruction and fragmentation, over-exploitation, pollution, climate change and movement of species into new locations).
2. The major genetic concerns in conservation biology are inbreeding depression, loss of genetic diversity, genetic drift overriding natural selection, population fragmentation, genetic adaptation to captivity and taxonomic uncertainties.
3. Inbreeding and loss of genetic diversity are inevitable in all small closed populations.
4. Inbreeding has deleterious effects on reproduction and survival (inbreeding depression) in almost every naturally outbreeding species that has been adequately investigated. Lower levels of inbreeding depression are typically found in naturally inbreeding species.
5. Loss of genetic diversity reduces the ability of populations to adapt (evolutionary potential) in response to environmental change. Quantitative genetic variation for reproductive fitness is the primary component of genetic diversity involved in adaptive changes.
6. Genetic factors generally contribute to extinction risk, sometimes having major impacts on persistence.
7. Ignoring genetic issues in the management of threatened species will often lead to sub-optimal management and in some cases to disastrous decisions.
8. The objective of genetic management is to preserve threatened species as dynamic entities capable of adapting to environmental change.
9. The first step in genetic management of a threatened species is to resolve any taxonomic uncertainties and to delineate management units within species. Studies using genetic markers can typically aid in resolving these issues.

10. Genetic management of wild populations is in its infancy and is not generally adequate or optimal to ensure long-term viability (largely because genetic issues are often ignored).
11. The greatest unmet challenge in conservation genetics is to manage fragmented populations to minimize inbreeding depression and loss of genetic diversity. Translocations among isolated fragments or creation of corridors for migration are required to minimize extinction risks, but they are being implemented in very few cases. Concerns about possible outbreeding depression (often exaggerated) have discouraged translocations to address the impacts of population fragmentation.
12. Captive breeding provides a means for conserving species that are incapable of surviving in their natural habitats. Captive populations of threatened species are typically managed to retain 90% of their genetic diversity for 100 years, using minimization of mean kinship.
13. Genetic deterioration in captivity resulting from inbreeding depression, loss of genetic diversity and genetic adaptations to captivity that are deleterious in the wild reduces the probability of successfully reintroducing species to the wild.
14. Population sizes of  $N_e$  much greater than 50 ( $N > 500$ ) are required to avoid inbreeding depression and  $N_e = 500\text{--}5000$  ( $N = 5000\text{--}50000$ ) are required to retain evolutionary potential. Many wild and captive populations are too small to avoid inbreeding depression and loss of genetic diversity in the medium term.
15. Population genomics has provided important insights into genome evolution, including allowing us to detect multilocus impacts, including selective sweeps, background selection and associative overdominance.
16. Invasive species have major adverse effects on biodiversity. Genetic factors affect the probability of species becoming invasive, their adaptations to local conditions, and the prospects of controlling them. Invasive species may also engender evolutionary responses in native species.
17. Molecular genetic analyses contribute to conservation by aiding forensic detection of illegal hunting and trade, and by providing

essential information on unknown aspects of species biology.

18. Genetic factors represent only one component of extinction risk. Wild populations face threats from both deterministic factors (habitat loss, over-exploitation, introduced species and pollution) that contribute to population declines, and stochastic factors (demographic and environmental stochasticity, catastrophes and genetic stochasticity) that become increasingly important in small populations. Genetic factors typically interact with other factors.
19. The combined impacts of all deterministic and stochastic threats faced by populations can be assessed using population viability analysis (PVA), typically done using stochastic computer projections. PVA is also used to carry out sensitivity analyses and to evaluate alternative management options to recover threatened species.
20. To be viable in the long term, wild populations typically have to consist of several thousand individuals.

We trust that you have found this book informative, thought-provoking and interesting and that it will assist in your future conservation activities. The Earth's biodiversity is being lost at a frightening rate, and we must act urgently to conserve our life-support system. We encourage you to participate in this wide-ranging activity.



## Revision problems

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**R.1** IUCN categories. In what category would you place the Lord Howe Island woodhen? It has a relatively stable population of about 160 and is restricted to Lord Howe Island (about 25 km<sup>2</sup>).

**R.2** Allele frequencies. What are the Hardy–Weinberg expected genotype frequencies at a locus with three alleles A, B and C at frequencies of 0.2, 0.3 and 0.5?

AA   AB   BB   AC   BC   CC   Total

**R.3** Linkage disequilibrium. Is the population with the following gametic frequencies in linkage equilibrium?

A <sub>1</sub> B <sub>1</sub>	A <sub>1</sub> B <sub>2</sub>	A <sub>2</sub> B <sub>1</sub>	A <sub>2</sub> B <sub>2</sub>
0.1	0.4	0.3	0.2

**R.4** Heritability. What is the heritability of shell breadth in the snail *Arianta arbustorum* given the following data on 119 pairs of parents and their offspring (after Hartl & Clarke [2007](#))?

Number of families	Mean of parents (mm)	Offspring mean (mm)
22	16.25	17.73
31	18.75	19.15
48	21.25	20.73
11	23.75	22.84
4	26.25	23.75
3	28.75	25.42

(The covariance between mid-parent and offspring is 5.183, and the variances for offspring and mid-parents are 3.311 and 8.180.)

**R.5** Mutation–selection equilibrium. What is the expected equilibrium frequency for the dominant D allele with a mutation rate of  $4 \times 10^{-6}$

and the following relative fitnesses?

DD	Dd	dd
0.7	0.7	1.0

**R.6** Time taken to regenerate genetic diversity by mutation. How many generations would it take for a microsatellite allele that had been lost from euros on Barrow Island, Western Australia to regenerate its previous frequency of 0.2, given a mutation rate of  $10^{-4}$  per gamete per generation?

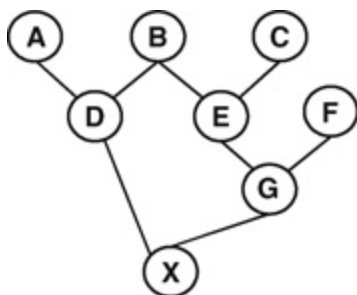
**R.7** Heterozygote advantage. What is the equilibrium frequency for the A allele, given the following survival values for the three genotypes (all genotypes have the same fertility)?

AA	Aa	aa
0.81	0.85	0.82

**R.8** Loss of genetic diversity in small populations. How much of the original heterozygosity would have been retained over 150 years in an outbreeding population of *Diprotodon* (extinct, cow-sized marsupial) with an effective size of 30 and a generation length of 15 years?

**R.9** Effective population size. What is the effective size of an insect population that fluctuates in size over three generations 100 000, 20, and 1000?

**R.10** Inbreeding. What is the inbreeding coefficient for individual X in the pedigree in the margin?



**R.11** Genetics of fragmented populations. What is  $F_{ST}$ ?

**R.12** Genetic distance. What is the genetic distance between two populations with the following allele frequencies?

	$A_1$	$A_2$	$A_3$
Woop Woop population	0.1	0.3	0.6
Outer Slobovia population	0	0	1.0

- R.13** Taxonomic uncertainties. Would you classify the two populations in Problem R.12 as separate species? Why?
- R.14** Genetic management. How do you go about genetically managing a clonally reproducing plant species?
- R.15** Genetic management. How are captive populations of threatened species managed by minimizing mean kinship?
- R.16** Genetic management. How large should a captive population be for the endangered okapi to maintain 90% of heterozygosity for 100 years, if the generation length is 7 years?

# Glossary

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## **Adaptive evolution**

Genetic change due to natural selection that improves the fitness of a population in its environment.

## **Additive**

Locus where the heterozygote has a mean phenotype exactly intermediate between the two homozygotes. Also referred to as additivity.

## **Additive variance**

That proportion of the genetic variation due to the average effects of alleles.

## **AFLP**

See *Amplified fragment length polymorphism*.

## **Allelic diversity**

Average number of alleles per locus, a measure of genetic diversity within a population.

## **Allelic dropout**

Failure of a microsatellite allele to amplify in some PCR reactions, especially when low-quality DNA is the template.

## **Allelic richness**

Allelic diversity standardized to a particular sample size.

## **Allopatric**

Populations or species whose distributions do not overlap.

## **Allopolyploid**

A species whose chromosomal complement derives from the full chromosome complements of two or more species e.g. autotetraploid. Many plant species have evolved via this form of instantaneous speciation.

**Allotetraploid**

A species whose chromosomal complement derives from the combination of two separate species (compare *Autotetraploid*).

**Allozygote**

An individual that is homozygous for two alleles that are not recent copies of the same allele, i.e. not identical by descent.

**Allozymes**

Alternative forms of a protein detected by electrophoresis that are due to alternative alleles at a single locus. Often referred to as *Isozymes*.

**Amino acids**

The building blocks of proteins.

**Amphidiploid**

An allopolyploid species that shows chromosomal segregation as if it were a diploid; for example bread wheat is a hexaploid with 42 chromosomes, but produces gametes with 21 chromosomes.

**Amplified DNA**

Many duplicated copies of a segment of DNA.

**Amplified fragment length polymorphism (AFLP)**

Method for detecting genetic variation across the genome by cutting genomic DNA with a restriction enzyme, adding short synthetic adaptor DNA fragments of known sequence to the cut ends, carrying out PCR using primers that match the adapter sequence and running amplified DNA on a gel. A multilocus DNA fingerprint is produced.

**Assignment test**

Assigning an individual to one of several populations, based upon highest relative probability of individual's genotype occurring in that population.

**Associative overdominance**

Heterozygote advantage due to linked alleles that are in linkage disequilibrium with each other and the studied locus.

**Autoradiography**

Detection of radioactively labelled molecules by their effects in

exposing photographic film.

**Autotetraploid**

A species derived by combining two full sets of chromosomes from the same species (compare *Allotetraploid*). One form of autopolyploidy.

**Autozygote**

An individual that is homozygous for two alleles that are recent copies of the same allele and have identical (or near identical) DNA sequences. Also referred to as *Identical by descent*.

**Backcross**

Cross of  $F_1$  progeny to one of the parent genotypes.

**Background selection**

A weak form of selection due to the removal of new deleterious mutations that may reduce genetic diversity at nearby loci in the process.

**Balancing selection**

Selection that maintains genetic variation in a population, encompassing heterozygote advantage (overdominance), rare advantage selection, and particular forms of selection that vary over space or time.

**Base population**

Population from which the studied population(s) were founded.

**Bayesian analysis**

Methods of analysis that incorporate other (prior) information.

**Binomial distribution**

The distribution describing the number of occurrences of two (or more) independent events in a sample of size  $n$ , e.g. the number of heads and tails in 50 tosses of a coin.

**Biodiversity**

Biological diversity; the variety of ecosystems, species, populations within species and genetic variation within living organisms.

**Biological control**

Use of introduced parasites, predators and pathogens to reduce invaders' density.

**Biological species concept**

Concept that species be defined by reproductive isolation. Gene flow is possible within, but weak or absent between species.

**Bioresources**

Products of use to humans that are obtained from the living world, e.g. food, fibre, timber and pharmaceutical drugs.

**Biparental inbreeding**

Inbreeding due to mating of relatives more remote than self (full-sibs, half-sibs, cousins, etc.).

**Bottleneck**

A sudden restriction in population size.

**Catastrophe**

An extreme environmental fluctuation that has a devastating impact on a population, e.g. cyclone, drought, extreme winter, disease epidemic, etc.

**CBSG**

Conservation Breeding Specialist Group of the Species Survival Commission of IUCN.

**cDNA**

Complementary DNA, often synthesized as a DNA copy of mRNA.

**Chloroplast DNA (cpDNA)**

Circular DNA molecules found in the chloroplasts of plants. They are usually maternally inherited.

**Cis**

Two DNA variants are in cis if they are on the same DNA molecule (or chromosome) and in trans if they are on different DNA molecules.

**CITES**

Convention on International Trade in Endangered Species of Wild Flora and Fauna.

**Clade**

A sub-group of organisms from among a larger group sharing common ancestry, not shared by the other organisms in the larger group.

**Cline**

Change in genetic composition of a population over a region, such as a latitudinal cline, or an altitudinal cline.

**Clone**

Individuals with identical genotypes, e.g. cuttings derived from a single plant, or several individual animals derived from a single animal by nuclear transplantation.

**Coalesce**

If two DNA sequence lineages converge at a common ancestor, they are said to coalesce.

**Coalescence**

The study of coalescence of DNA sequence lineages.

**Coalescent theory**

Investigations of the mathematical and statistical properties of genealogies (see *Gene trees*).

**Coancestry**

The coancestry to two individuals is the probability that two alleles, one from each individual, are identical by descent. Synonymous with *Kinship*.

**Co-dominance**

The condition where the heterozygotes at a locus are distinguishable from both homozygotes.

**Coefficient of linkage disequilibrium ( $D$ )**

A measure of the non-random association of alleles at different loci. If the gametic types and frequencies are as follows:

$$\begin{array}{cccc} A_1B_1 & A_1B_2 & A_2B_1 & A_2B_2 \\ r & s & t & u \end{array}$$

then  $D = ru - st$

**Common ancestor**



An individual that is an ancestor of both the mother and the father of a particular individual.

**Common garden experiment**

Comparison of different genotypes in the same environment to distinguish genetic differences from environmental ones, usually for quantitative characters.

**Conspecific**

Belonging to the same species.

**Convergent evolution**

Evolution of similar phenotypes in distinct species subjected to similar environmental conditions, e.g. similar adaptations to marine environments in fish and in marine mammals and reptiles.

**Corridor**

Ribbon of habitat between population fragments.

**cpDNA**

See *Chloroplast DNA*

**Critically endangered**

A species with a very high probability of extinction within a short time, e.g. 50% probability of extinction within 10 years, or three generations, whichever is longer.

**Demographic stochasticity**

Fluctuation in birth and death rates and sex-ratio due to chance alone that may drive a small population to extinction.

**Demography**

The study of how vital rates, such as fecundity, survival and migration, influence population growth and persistence.

**Dioecious**

Having separate sexes, especially in plants.

**Directional selection**

Selection in which the most extreme high (or low) individuals from a population are chosen as parents of the next generation.

**Disruptive selection**

Selection of varying direction within the range of a species, e.g. favoured melanic peppered moths in polluted areas, but non-melanic peppered forms in non-polluted areas.

**DNA barcoding**

Use of short DNA sequences, such as the COI region of mtDNA, to help distinguish taxonomic units, discover new species and to assign unidentified individuals to species.

**DNA fingerprint**

The 'barcode' produced by probing for minisatellites on the DNA of an individual. Also called variable number tandem repeats (VNTR).

**Dominance**

Deviation of heterozygote phenotype from the mean of homozygotes at a locus.

**Dominance variance**

That proportion of the quantitative genetic variation due to the deviation of heterozygotes from the average effects of the two homozygotes.

**Ecosystem services**

Essential functions supplied free of charge by living organisms, including oxygen production from green plants, nutrient recycling, pest control and pollination of crop plants.

**Ecotype**

Populations within a plant species that are genetically adapted to different ecological conditions, often of soil and climate.

**Effectively neutral**

The situation where the selective forces on an allele are so weak that it behaves as if it is not subject to natural selection. Occurs when the selection coefficient is less than  $\sim 1/(2N_e)$ , where  $N_e$  is the effective population size.

**Effective number of alleles ( $n_e$ )**

The number of alleles that if equally frequent would result in the

observed heterozygosity.

**Effective population size ( $N_e$ )**

The number of individuals that would result in the same loss of genetic diversity, inbreeding or genetic drift if they behaved in the manner of an idealized population.

**Electrophoresis**

A method for separating proteins or DNA fragments in a gel according to their net charges, shape and sizes.

**Endangered**

A species or population with a high probability of extinction within a short time, e.g. a 20% probability of extinction within 20 years, or 10 generations, whichever is longer.

**Endemic**

A population or species found in only one region or country.

**Environmental stochasticity**

Effect of natural fluctuations in environmental conditions, such as rainfall, food supply, competitors, winter temperatures, etc. that affect a species, and may drive a small population to extinction.

**Epistasis**

Interactions among gene loci in their effects on phenotype.

**Epistatic variance**

That proportion of the quantitative genetic variation due to the deviation of genotypic effects from the average effects of the constituent loci. Synonymous with *Interaction variance*.

**Equilibrium**

State where a population or other entity has no tendency to change from its present condition across time.

**ESU**

See *Evolutionarily significant units*.

**Evolution**

Change in the genetic composition of a population.

**Evolutionary potential**

The ability of a population to evolve to cope with environmental changes. Often simplistically equated with genetic diversity.

**Evolutionarily significant units (ESU)**

Partially genetically differentiated populations that justify management as separate units.

**Exon**

Region of a functional locus that is transcribed and translated, e.g. DNA sequence that specifies amino acids in a protein.

**Expected heterozygosity ( $H_e$ )**

The heterozygosity expected for a random mating population with the given allele frequencies according to the Hardy–Weinberg equilibrium.

***Ex situ***

Away from its normal habitat, such as an endangered species being conserved in captivity.

**Extinction**

Permanent disappearance of a population or species.

**Extinction vortex**

Describes the likely adverse interaction between human impacts, inbreeding and demographic fluctuation that result in a feedback loop and spiral towards extinction.

 **$F$** 

Wright's inbreeding coefficient. Probability that two alleles at a locus in an individual are identical by descent.

 **$F_{IS}$** 

That proportion of the total inbreeding within a population due to inbreeding within sub-populations.

 **$F_{IT}$** 

The total inbreeding in a population, due to both inbreeding within sub-populations, and differentiation among sub-populations.

**Fitness**

Reproductive fitness, the number of fertile offspring contributed by an individual that survive to reproductive age.

**Fixation**

All individuals in a population being identically homozygous for a locus, e.g. all  $A_1A_1$ .

**Forensics**

Application of science to the law, including detection of illegal activities by scientific means.

**Founder effect**

Change in the genetic composition of a population due to origin from a small sample of individuals. Typically results in loss of genetic diversity, genetic drift and increased inbreeding.

**Frequency dependent selection**

A form of natural selection where the relative fitnesses of genotypes vary with their frequencies.

 **$F_{ST}$** 

The proportion of the total inbreeding in a population due to differentiation among sub-populations.

 **$F$  statistics**

Measures of total inbreeding in a population ( $F_{IT}$ ), partitioned into that due to inbreeding within sub-populations ( $F_{IS}$ ) and that due to differentiation among sub-populations ( $F_{ST}$ ).

**Full-sib mating**

A mating between a brother and a sister.

**Gene diversity**

See *Expected heterozygosity*.

**Gene drop**

Computer simulation of the fate of alleles due to Mendelian segregation in a pedigree.

**Gene flow**

Movement of alleles between populations via migrants.

**Gene genealogies**

Tree diagrams showing the relationships between different copies of a single locus (gene trees).

**Gene trees**

Tree diagrams showing the relationships between different copies of a single locus (gene genealogies), typically devised on the basis of DNA sequences.

**Genetic distance**

A measure of the genetic difference between allele frequencies in two populations or species, e.g. Nei's genetic distance.

**Genetic diversity**

The extent of genetic variation in a population or species, or across a group of species, e.g. heterozygosity, or allelic diversity, or heritability.

**Genetic drift**

Changes in the genetic composition of a population due to random sampling in finite populations. Also referred to as *Random genetic drift*.

**Genetic erosion**

Inbreeding depression and loss of genetic diversity in small populations.

**Genetic load**

The content of deleterious alleles in a population, some due to mutation–selection balance (mutation load) and others to heterozygote advantage and other forms of balancing selection (balanced load).

**Genetic rescue**

Improvement in reproductive fitness and increase in genetic diversity due to outcrossing of a population previously suffering low genetic diversity and inbreeding.

**Genetic stochasticity**

Genetic consequences of small populations, including inbreeding, loss of genetic diversity and mutational accumulation, that may drive a population or species to extinction.

**Genome**

The entire DNA or all of the chromosomes in an individual or a species.

**Genome-enabled taxa**

A species or sub-species that is taxonomically closely related to one whose DNA has been sequenced.

**Genome resource bank**

A storage facility containing genetic material for several species, including seed stores, cryopreserved gametes, embryos, or somatic cells, or a collection of DNA samples.

**Genotype × environment interaction**

Differential performance of diverse genotypes in dissimilar environments.

**Gynodioecious**

Species containing some plants with female flowers and others with hermaphroditic flowers.

**Haplotype**

Allelic composition for several different loci in a chromosomal region, e.g.  $A_1B_3C_2$ .

**Haplotype diversity**

A measure of genetic diversity among haplotypes.

**Haplotype network**

A diagram showing different haplotypes joined by lines to show relationships, typically based on DNA sequences.

**Hardy–Weinberg equilibrium**

The equilibrium genotype frequencies achieved in a random mating population with no perturbing forces from mutation, migration, selection or chance. If two alleles  $A_1$  and  $A_2$  have frequencies of  $p$  and  $q$ , the Hardy–Weinberg equilibrium frequencies for the  $A_1A_1$ ,  $A_1A_2$  and  $A_2A_2$  genotypes are  $p^2$ ,  $2pq$  and  $q^2$ , respectively.

**Harmonic mean**

Reciprocal of the arithmetic mean of reciprocals =  $n / \Sigma(1/X_i)$

**Heritability ( $h^2$ )**

Proportion of the variation for a quantitative character due to genetic causes.

**Hermaphrodite**

An animal or plant with both sexes present in single individuals. Referred to as monoecious in plants.

**Heterosis**

Hybrid vigour. Superior performance of hybrid genotypes, usually indicating superiority to both parental genotypes.

**Heterozygosity**

Proportion of heterozygous individuals for a locus in a population.

**Heterozygote advantage**

A form of selection where the heterozygote has a higher reproductive fitness than the homozygotes. Also referred to as *Overdominance*.

**Homoplasy**

Apparently similar alleles with different DNA sequences, as for allozyme or microsatellite alleles.

**Idealized population**

A conceptual random mating population with equal numbers of hermaphrodite individuals breeding in each generation, and Poisson variation in family sizes (mean = variance = 2). Used as a standard to which other populations are equated when defining effective population sizes.

**Identity by descent**

Alleles that are identical copies of an allele present in a common ancestor.

**Inbreeding**

The production of offspring from mating of individuals related by descent, e.g. self-fertilization, brother  $\times$  sister, or cousins matings.

**Inbreeding coefficient ( $F$ )**



The probability that two alleles at a locus in an individual are identical by descent. Used to measure the extent of inbreeding.

**Inbreeding depression**

Reduction in mean for a quantitative trait due to inbreeding, especially in reproduction or survival.

**Indel**

An insertion or deletion mutation, typically of a few bases or of a transposable element.

***In situ* conservation**

Conservation of a species or individual in its normal wild habitat.

**Interaction variance**

The proportion of quantitative genetic variation due to the deviation of genotypic effects from the average effects of the constituent loci. Synonymous with *Epistatic variance*.

**Introgression**

Introduction of genetic material from another species or sub- species into a population. A threat to the genetic integrity of a range of canid, fish, plant, etc., species.

**Intron**

Region of a locus that is transcribed, but not translated.

**Invasive species**

A species that introduces into a new range, establishes and spreads, typically invading large areas and adversely affecting many other species.

**Inversion**

A chromosome aberration in which a region of chromosome has been turned through 180 degrees, such that gene order is changed, say from ABCDE to ADCBE.

**Isozymes**

Alternative forms of a protein detected by electrophoresis.

**IUCN**

The World Conservation Union. The initials originally stood for the International Union for the Conservation of Nature.

**Kinship ( $k_{ij}$ )**

The kinship of two individuals is the probability that two alleles, one from each individual, are identical by descent. Synonymous with *Coancestry*.

**Landscape genetics**

Study of patterns of genetic diversity and gene flow across landscapes.

**Lethal**

Inconsistent with survival, as in a recessive lethal allele that results in death when homozygous.

**Lethal equivalents ( $B$ )**

A measure for comparing the extent of inbreeding depression in different populations. A group of detrimental alleles that would cause death if homozygous, e.g. one lethal allele, two alleles each with a 50% probability of causing death, etc. Estimated from the slope of the regression of  $\ln(\text{survival})$  on the inbreeding coefficient  $F$ .

**Lineage sorting**

Random loss of genetic variants in different lineages deriving from a polymorphic common ancestral species (or population).

**Linkage disequilibrium**

Non-random association of alleles at different loci.

**Locus**

A segment of DNA on a chromosome.

**Major histocompatibility complex (MHC)**

A large family of loci that play an important role in the vertebrate immune system and in fighting disease.

**Management unit**

Populations within a species that are sufficiently distinct to require separate genetic management.

**Maternal effect**

Effect of maternal environment on phenotype of an offspring, e.g. effect on offspring weight of nutrient supply from mother.

**Maximum likelihood**

Statistical method used to obtain the estimate of a parameter that maximizes the probability of the observed result.

**Mean kinship ( $mk_i$ )**

The average kinship of an individual with all individuals in a population, including itself. Minimizing mean kinship is the recommended method for genetically managing endangered species in captivity.

**Meta-analysis**

A statistical analysis that uses the combined information from several different studies or species.

**Metapopulation**

A group of partially isolated populations of the same species that undergo local extinctions and recolonizations.

**MHC**

See *Major histocompatibility complex*.

**Microarray**

A slide spotted with many short single-stranded DNA sequences, suitable either for genotyping or for measuring levels of mRNA.

**Microsatellite**

A locus with a short tandem repeat DNA sequence, such as the AC sequence repeated 10 times. Such loci typically show variable numbers of repeats and high heterozygosities in populations.

**Migration**

Gene flow between populations.

**Minimum viable population size (MVP)**

The minimum size of population that will be viable in the long term, meaning a probability of persistence of say 99% for 40 generations, or 90% for 100 years.

**Minisatellite**

A region of DNA, usually in the 10s to 100s of bases in length that show variation in number of repeats. Also known as variable number tandem repeats (VNTR). When several such loci are probed they result in a DNA fingerprint that looks like a barcode.

**miRNA**

MicroRNAs are short non-coding RNAs, found in many plants and animals, that often regulate gene expression post-transcriptionally.

**Mitochondrial DNA (mtDNA)**

The circular DNA molecules contained within mitochondria. Usually maternally inherited.

**Molecular clock**

Concept that the extent of molecular genetic divergence is related to time. Used to date biological events.

**Molecular operational taxonomic unit (MOTU)**

Taxonomic unit defined purely from distinction at the molecular level, e.g. by DNA barcoding. May or may not be a distinct species.

**Monomorphic**

A locus at which only one allele is present, generally taken to mean the most common allele is at a frequency of greater than 99%, or 95%. Contrast with *Polymorphic*.

**Monophyletic**

A group of species (or DNA sequences) that derive from the same common ancestral species (or DNA sequence). Converse is *Polyphyletic*.

**MOTU**

See *Molecular operational taxonomic unit*.

**mtDNA**

See *Mitochondrial DNA*.

**Mutation**

A sudden genetic change, i.e. parents lack the condition, but it appears in one or more offspring.

**Mutation load**

Deleterious mutations carried in a population.

**‘Mutational meltdown’**

Decline in reproductive rate and downward spiral towards extinction due to chance fixation of new mildly deleterious mutations in small populations.

**Mutation–selection balance**

The equilibrium between the occurrence of deleterious mutation and natural selection removing them, resulting in low frequencies of deleterious mutations in populations.

**MVP**

See *Minimum viable population size*.

**Natural selection**

Mortality or altered reproduction due to natural environmental processes.

**ncRNA**

Small regulatory non-coding RNA molecules.

**Nei's genetic distance ( $D_N$ )**

The most widely used measure of the genetic difference between allele frequencies in two populations or species, devised by Masatoshi Nei.

**Nei's genetic similarity ( $I_N$ )**

The most widely used measure of the genetic similarity between allele frequencies in two populations, or species, devised by Nei.

**Neutral mutation**

A mutation that is equivalent in effects on reproductive fitness to the existing allele.

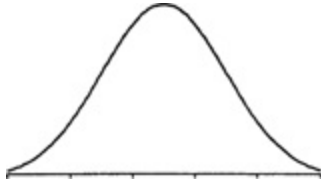
**Non-synonymous substitution**

A base substitution at a locus that results in a change in amino acid composition of its protein. Compare *Synonymous substitution*.

**Normal distribution**

A symmetrical bell-shaped distribution with a characteristic mean and

variance as shown in the margin. 95% of values lie within 1.96 standard deviations of the mean. Many quantitative characters show approximately normal distributions.

**Nucleotide diversity ( $\pi$ )**

A measure of genetic diversity at the nucleotide level. Heterozygosity at the nucleotide level in a random mating population.

**Null allele**

Allele that does not produce a functional product, or a mutation in a primer site that precludes PCR amplification.

**Outbred**

An individual whose parents are unrelated.

**Outbreeding**

Not inbreeding. Approximately random mating.

**Outbreeding depression**

A reduction in reproductive fitness due to crossing of two populations (or sub-species, or species).

**Outcrossing**

Crossing a population to another that is not closely related.

**Overdominance**

Heterozygote advantage; a form of selection where heterozygotes have a higher fitness than homozygotes.

**Panmictic**

Random mating.

**Partial dominance**

The condition where the heterozygote has a phenotype closer to one homozygote than the other, i.e. heterozygotes for most deleterious alleles are nearly but not completely normal.

**PCR**

See *Polymerase chain reaction*.

**Pedigree**

A chart specifying lines of descent and relationship among individuals.

**Peripheral character**

A character with limited relationship to reproductive fitness, e.g. bristle number in fruit flies or tail length in rodents.

**Phenotypic plasticity**

Variation in the phenotype of individuals with the same genotype due to their experiencing different environments, e.g. haemoglobin levels in people living at different altitudes.

**PHVA**

See *Population and habitat viability assessment*.

**Phylogenetic tree**

A tree diagram representing the closeness of relationship between species or populations.

**Phylogeography**

Study of the geographic distribution of genealogical lineages, especially within species, e.g. a DNA sequence tree where haplotypes are mapped onto geographic locations.

**Poisson distribution**

A probability distribution, with mean = variance, used to predict the number of occurrences of rare independent events, such as the number of individuals carrying 0, 1, 2, etc. new mutations, or the distribution of families of sizes 0, 1, 2, 3, . . .

**Polyandry**

Mating system in which females produce offspring from several males.

**Polygamy**

Mating with more than one of the opposite sex.

**Polygene**

More commonly referred to as a *Quantitative trait locus*.

**Polygyny**

Mating system in which males mate with several females, i.e. harems.

**Polymerase chain reaction (PCR)**

Method used to make replicate copies (amplify) of a specific segment of DNA. The DNA is heated, primers (short segments of DNA flanking the segment of interest) added and the intervening DNA copied over 30–40 cycles using thermostable *Taq* polymerase enzyme.

**Polymorphic**

A locus at which more than one allele is present, generally taken to mean the most common allele is at a frequency of less than 99%, or 95%. Compare with *Monomorphic*.

**Polyphyletic**

A group of species (or DNA sequences) that derives from more than one ancestral species (or DNA sequence).

**Polyploid**

Having more than two doses of each chromosome, e.g. tetraploid ( $4n$ ).

**Population and habitat viability assessment (PHVA)**

A workshop process developed by CBSG that encompasses a population viability analysis that includes the impacts of habitat changes.

**Population genomics**

The study of variation and evolution of genomes.

**Population viability analysis (PVA)**

The process of predicting the fate of a population (including risk of extinction) due to the combined effects of all deterministic and stochastic threats faced by a population. Typically population size, means and standard deviation of birth and death rates, plus risks and severity of catastrophes, levels of inbreeding depression, etc. are input into a software package and many replicates projected over several generations using stochastic computer simulation. Used as a management and research tool in conservation biology.

**Primer**



A short nucleotide sequence that pairs with one strand of DNA and provides a free end at which DNA polymerase enzyme begins synthesis of a complementary segment of DNA.

**Probe**

DNA from a known locus used to hybridize with other DNA via complementary base pairing to identify similar sequences in the other DNA.

**Pseudogene**

A non-functional copy of a locus.

**Purging**

Elimination of deleterious alleles from populations due to natural selection, especially that associated with populations subject to inbreeding.

**Purifying selection**

Selection that removes deleterious alleles and maintains a current constrained functional DNA sequence.

**PVA**

See *Population viability analysis*.

**QTL**

See *Quantitative trait locus*.

**Quantitative character**

Typically a trait with a continuous distribution influenced by genetic and environmental variation, e.g. height, weight, fecundity and survival.

**Quantitative genetic variation**

Genetic variation affecting a quantitative character, such as size, reproductive rate, behaviour or chemical composition. Presumed to be due to the cumulative effects at many loci (QTL). Also referred to as polygenic variation.

**Quantitative trait locus (QTL)**

A locus affecting a quantitative character. Also referred to as a *Polygene*.

**Random genetic drift**

Changes in the genetic composition of a population due to random sampling in finite populations.

**Randomly amplified polymorphic DNA (RAPD)**

Genetic diversity detected following PCR amplification using random primers of DNA (usually 10 or more bases in length) to amplify random segments of DNA. Results in a multilocus DNA fingerprint, akin to a barcode.

**Random mating**

A pattern of mating where the probability of genotype formation is determined by allele frequencies in the population.

**RAPD**

See *Randomly amplified polymorphic DNA*.

**Rare advantage selection**

A form of balancing selection in which alleles are favoured when rare, but selected against when common, e.g. for self-incompatibility alleles.  
A form of frequency-dependent selection.

**Reintroduction**

Returning a species or population to part of its former range using individuals from captive populations.

**Relative fitness**

The fitness of a genotype, compared to another genotype, usually at the same locus. For example, if fitness of genotypes at a locus conferring warfarin resistance are 30%, 80% and 54% for RR, RS and SS, then their relative fitnesses are  $30/80 = 0.375$ ,  $80/80 = 1$  and  $54/80 = 0.68$ , respectively.

**Reproductive fitness**

The number of fertile offspring surviving to reproductive age contributed by an individual. Often referred to as *Fitness*.

**Restriction enzyme**

An enzyme that cuts DNA at points determined by specific DNA recognition sequences of various lengths, e.g. 4, 6 or 8 bases.

**Restriction fragment length polymorphism (RFLP)**

Genetic diversity detected by cutting DNA with restriction enzymes.

**RFLP**

See *Restriction fragment length polymorphism*.

**RNAi**

Small interfering RNA molecules involved in defences against virus infections and transposons.

**Selection coefficient (s)**

The difference in relative fitness between a genotype at a locus and the one with the highest fitness. For example, if three genotypes  $A_1A_1$ ,  $A_1A_2$  and  $A_2A_2$  have relative fitnesses of 1, 1 and 0.9, the selection coefficient for the  $A_2A_2$  genotypes is  $s = 1 - 0.9 = 0.1$ .

**Selection differential (S)**

A measure of the intensity of selection on a quantitative character – the difference in mean between the selected parents and the mean of the total population from which they derived.

**Selectively neutral**

An allele whose fate is determined by chance sampling in a small population as the selective forces on it are weak in relation to the effects of chance, defined as  $s < 1/(2N_e)$ .

**Selective sweep**

Action of natural selection driving a single allele to fixation, and at the same time reducing genetic diversity for surrounding DNA (often neutral to selection themselves).

**Self-incompatibility**

The inability of an individual (usually plant) to produce offspring following attempted self-fertilization. Many plant species have loci that control self-incompatibility.

**Selfing**

Self-fertilizing.

**Self-sterility**

See *Self-incompatibility*.

**Sensitivity analysis**

The set of analytical and simulation-based tools that evaluate how changes in specific life history attributes, habitat quality, predation, etc. affect population growth or extinction risk for a species.

**Sex-linked**

A locus found on the sex chromosomes (X in mammals, Z in birds and Lepidoptera), such that there is unequal transmission from the two sexes of parents to offspring of different sexes.

**Sibling species**

Two or more closely related species that are morphologically similar.

**Silent substitution**

A DNA base substitution that does not alter the amino acid composition of a polypeptide chain.

**Single large or several small (SLOSS)**

The concept that compares the consequences of a single large population versus several small populations of equivalent total size, especially in terms of their extinction proneness.

**Single nucleotide polymorphism (SNP)**

A position in the DNA of a species at which two or more alternative bases occur at appreciable frequency ( $> 1\%$ ).

**siRNA**

Small interfering double-stranded RNA molecules that are involved in RNA interference and other regulatory functions.

**SLOSS**

See *Single large or several small*.

**SNP**

See *Single nucleotide polymorphism*.

**Source–sink**

A population structure where one population, the source, is permanent and supplies individuals to restart one or more transient sink

populations.

**Southern blot**

Transfer of DNA from a gel to a membrane by blotting, such that liquid is drawn from the gel through the membrane, with the DNA transferred at the same time from the gel to the membrane.

**Spatial autocorrelation**

A statistical method that tests whether individuals that are adjacent to each other in space are more similar than those further apart.

**Speciation**

The processes by which populations diverge and become reproductively isolated so that they develop into different species.

**Species**

Mayr defined species as 'groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups' according to the biological species concept.

**Stabilizing selection**

Selection favouring phenotypic intermediates at the expense of phenotypic extremes.

**Stable equilibrium**

Allele frequency to which the population returns no matter what direction the frequency is perturbed.

**Statistical power**

The ability to reject an erroneous null hypothesis.

**Stochastic**

Having a chance element. Having variable outcomes described by a probability distribution, e.g. environmental and demographic stochasticity, genetic drift.

**Studbook**

A record of the pedigrees of ancestors and living individuals in a population.

**Sub-species**

Taxonomic units within species that are partially genetically differentiated populations.

**Supportive breeding**

Regular augmentation of a wild population with individuals from a captive population.

**Sympatric**

Populations that share the same or overlapping distributions.

**Synonymous substitution**

A base substitution at a locus that does not result in a change in amino acid composition of a protein specified by the locus. Contrast with *Non-synonymous substitution*.

**Tandem repeats**

Multiple copies of the same sequence lying one after another in a series, as in microsatellite repeats, or minisatellite repeats.

**Taxa**

Several populations belonging to a taxonomic unit, e.g. several species, or several sub-species, etc. Singular: taxon.

**Telomere**

Short tandem DNA repeats at the ends of eukaryotic chromosomes that stabilize them.

**Tetraploid**

Species with four doses of each chromosome.

**Threatened**

A population or species that has a finite risk of extinction within a relatively short time frame, say a greater than 10% risk of extinction within 100 years. Under the IUCN system this is the sum of the critically endangered, endangered and vulnerable categories.

**Transcriptome**

The array of transcripts from the genome.

**Transgene**

Locus genetically engineered into a species or individual from another.

**Transient polymorphism**

The temporary state where a locus is polymorphic, as when a favourable mutation rises in frequency towards eventual fixation, or a neutral allele drifts in a population.

**Translocation**

The movement of an individual from one wild location to another as a result of human actions.

**Transposons**

Mobile genetic elements found in species from bacteria to mammals.

**Trans-species polymorphism**

An ancient polymorphism where related species share similar polymorphic alleles at a locus. For example, these exist at MHC and self-incompatibility loci and are maintained by balancing selection.

**UTR**

Untranscribed region of the genome, as in 3' UTR.

**Variance**

The most commonly used measure of dispersion among quantitative measurements. The square of the standard deviation. The summed squared deviations from the mean divided by  $(n-1)$ .

**VNTR**

Variable number tandem repeat. see *DNA fingerprint*.

**Vulnerable**

A species or population with a tangible risk of extinction within a moderate time, e.g. a 10% probability within 100 years.

**Wahlund effect**

Reduction in heterozygosity, compared to Hardy–Weinberg expectations, in a population split into partially isolated sub-populations. Named after its discoverer.

## References

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(For a given author, single author papers are first, followed by papers with two authors, and finally papers with three or more authors in year order.)

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# Index

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Page numbers in **bold** refer to figures; page numbers in *italics* refer to tables.

- achondroplasia [148](#)
- Acinonyx jubatus* *see* [cheetah](#)
- acorn woodpecker (*Melanerpes formicivorus*) [509](#)
- Acrocephalus sechellensis* *see* [Seychelles warbler](#)
- adaptive evolution
  - animals [117](#)
  - cyanogenic glucosides [117](#)
  - definition [117](#), [527](#)
  - industrial melanism [124](#)
  - plants [117](#)
  - transcription levels [222](#)
- adaptive genetic diversity, captive population management [446](#)
- adder (*Vipera berus*), inbreeding depression [290](#)
  - genetic rescue [306](#), [306](#)
- additive, definition [527](#)
- additive dominance [127](#)
- additive genetic variance ( $V_A$ ) [97](#), [98](#), [99](#)
- additive loci [98](#), [98](#)
- additive variation [95](#)
  - definition [527](#)
- advantageous mutations [146](#)
- Aepyceros melampus*
  - see also* impala
- AFLP *see* [amplified fragment length polymorphisms \(AFLP\)](#)
- African black-necked cobra (*Naja nigricollis*) [352](#)
- African lion (*Panthera leo*), genetic diversity [43](#), [45–46](#)
- aging, forensics [493](#)
- Ailuropoda melanoleuca* *see* [giant panda](#)

- albinism [148](#)
- Alces alces* (moose) [273](#), [406](#)
- alcohol dehydrogenase, *Drosophila* [57](#)
- allele(s) [44](#)
  - deleterious see [deleterious alleles](#)
  - loss in small populations [30](#)
  - recessive see [recessive alleles](#)
- allele diversity (A) [73–74](#)
  - bottlenecks [172](#)
  - definition [44](#), [527](#)
- allele frequencies
  - definition [44](#)
  - directional selection [217](#)
  - generation number [130–131](#)
  - genetic diversity [67–69](#)
  - inbreeding [267](#)
  - MHC polymorphism [194](#)
- models
  - genetic drift [165–168](#), [166](#)
  - migration models [153](#), [154](#), [154–155](#)
  - sampling [166](#), [166](#)
- multigeneration effects [318](#), [319](#)
- mutation load [148](#)
- recessive alleles [76](#)
- allelic diversity [43](#)
- allelic dropout [527](#)
  - DNA-based censoring [478](#)
- allelic richness [74](#), [527](#)
- Allen's rule [155](#)
- allopatric speciation [370](#), [373–375](#)
  - definition [527](#)
  - genetic markers [379](#)
  - sympatric speciation vs. [374](#)
- allopolyploidy [527](#)
  - speciation [371](#), [371](#)
- allotetraploids [527](#)



allozygous, definition [263](#), [527](#)  
allozymes [527](#)  
    genetic diversity measurement [54](#)  
*Amazona vittata* see [Puerto Rican parrot](#)  
American chestnut (*Castanea dentata*) [398](#)  
American oyster (*Crassostrea virginica*) [487](#)  
amino acids [527](#)  
    neutral mutations under random genetic drift [186](#), [186](#)  
*Ammodramus maritimus nigrescens* see [dusky seaside sparrow](#)  
amphibians, projected extinction rates [5](#)  
amphidiploid [527](#)  
amplified DNA [527](#)  
amplified fragment length polymorphisms (AFLP) [527](#)  
    genetic diversity measurement [50–51](#), [54](#)  
angiosperms, inbreeding depression [296](#)  
animal(s)  
    adaptive evolution [117](#)  
    cloning [448](#)  
    exchange, captive population management [461](#)  
Animal Record Keeping System (ARKS) [432](#)  
antechinus, population fragmentation [329](#)  
*Anthyllis vulneria* (kidney vetch), isolation by distance [331](#)  
Apalachicola rosemary (*Conradina glabra*) [344](#)  
Arabian oryx (*Oryx leucoryx*)  
    bottlenecks [169](#)  
    captive breeding [435](#)  
    heterozygosity vs. generation time [352](#)  
    reintroduction [466](#)  
*Arabidopsis thaliana*  
    coding loci numbers [213](#)  
    reproductive fitness [93](#)  
    self-fertilization [408](#)  
Argentine ant (*Linepithema humile*) [415](#), [419](#)  
*Argyroxiphium sandwicense macrocephalum* see [Mauna Kea](#)  
[silversword](#)  
ARKS (Animal Record Keeping System) [432](#)

ARLEQUIN [87](#), [335](#)  
artificial insemination (AI) [447](#)  
Aruba island rattlesnake (*Crotalus durissus unicolor*) [344](#)  
asexual species, wild population management [407–408](#)  
Asian elephant (*Elephas maximus*)  
    harvesting impacts [406](#), [406](#)  
    unequal sex-ratio [250](#)  
Asian flamegold tree (*koelreuteria elegans*) [420](#)  
Asian rhinoceros see [Indian rhinoceros \(\*Rhinoceros unicornis\*\)](#)  
Asian shore crab (*Hemigrapsus sanguineus*) [420](#)  
Asiatic lion (*Panthera leo persica*)  
    reproductive success/family size [246](#), [247](#)  
    wild population sizes [343](#)  
Assateague Island horses [421](#), [422](#)  
assignment tests [527](#)  
associative overdominance [527](#)  
    small population genetic diversity maintenance [202–204](#), [203](#)  
*Astrocaryum mexicanum*  
    see also Choco palm  
at-risk species identification [10](#)  
Attwater's prairie chicken (*Tympanuchus cupido attwateri*) [351](#)  
Australia, myxomatosis, introduction of [421](#)  
Australian brushtail possums (*Trichosurus vulpecula*) [421](#)  
Australian sheep blowfly (*Lucilia cuprina*) [423](#)  
autopolyploidy, speciation [371](#)  
autoradiography, definition [527](#)  
autosomal loci  
    compound, invasive species control [423](#), [424–425](#)  
    dominance [128](#), [129](#)  
    equilibrium frequencies [149](#)  
autotetraploids  
    definition [527](#)  
    segregation patterns [281](#)  
    selfing [281](#)  
autozygous [527](#)  
*Avena* (wild oats), inbreeding [266](#)

average heterozygosity ( $H$ ) 69

definition 44

avian malaria 116, 417, 494

backcrossing 277, 278, 278–279

definition 527

inbreeding increase 276, 277, 278–279

recurrence relationships 277

background selection 217, 218, 528

bacteria 411

*Balaenoptera physalus*

see also fin whales

balancing selection 184, 188, 189–199

definition 528

heavy-metal tolerance 197

heterogenous environments 197

heterozygote advantage 189–190, 189–192, 191, 191–192

industrial melanism 197

rare allele advantage 192–195

reproductive fitness 198–199

resistance to 195

small population genetic diversity maintenance 199

see also specific types

bald eagle (*Haliaeetus leucocephalus*) 351

Bali starling (*Leucopsar rothschildi*) 344

ballast water treatment, invasive species control 420

balloon vine (*Cardiospermum grandiflorum*) 420

*Banksia brownii* (Brown's banksia) 276

*Banksia cuneata*

see also matchstick banksia

BAPS 335

base population

definition 528

inbreeding measurement 265

Bay checkerspot butterflies (*Euphydryas editha bayensis*) 118, 504–505,

- Bayesian analysis 528
- B blood group alleles, migration 153, 153
- bears, isolation by distance 331
- BEAST 388
- behavioral modification 116
- beneficial mutations 183
  - removal 183
- Bergmann's rule 155
- Bettongia penicillata*
  - see also brush-tailed bettong
- Bidens amplexans*
  - see also kokolau
- bighorn sheep (*Ovis canadensis*)
  - isolation by distance 331, 331
  - population size vs. persistence 24
- bilby (*macrotis lagotis*) 119
- binomial distribution 528
- biocide resistance, invasive species control 423
- biocontrol agents, resistance evolution 119
- biodiversity
  - conservation importance 2–3
  - definition 2, 528
  - invasive species 415–416
- biological control 421, 458
  - definition 528
  - invasive species control 419–420, 421
- biological species concept
  - definition 528
  - species definition 367
- bioresources 2, 528
- biparental inbreeding
  - definition 528
  - forensics 488
- birds
  - dispersal distances 330
  - inbreeding depression 287

- population viability [291](#)
- molecular sexing [492](#), [492](#)
- projected extinction rates [5](#)
- sexing of [13](#)
- see also individual species*
- birth weight, stabilizing selection [135](#), [136](#)
- bison (*Bison*) [483](#)
- Bison bonasus* *see* [European bison](#)
- black-footed ferret (*Mustela nigripes*)
  - bottlenecks [169](#)
  - captive breeding
    - effective population size [354](#)
    - founder numbers [435](#)
  - genetic diversity loss [237](#)
  - population targets [351](#)
  - population viability analysis [510–511](#)
  - reintroduction [466–467](#)
  - reproductive fitness [93](#)
- black-footed rock wallabies (*Petrogale lateralis*)
  - genetic rescue [398](#)
  - inbreeding depression [26](#), [26](#)
  - management [355](#)
  - population fragmentation [315](#), [318](#)
    - homozygosity [79](#)
- black rhinoceros (*Diceros bicornis*)
  - migration corridors [400](#), [401](#)
  - taxonomy [387](#)
- black snake, red-bellied (*Pseudechis porphyriacus*) [420](#)
- BLAST [227](#)
- blue mussel (*Mytilus edulis*) [420](#)
- Boiga irregularis*
  - see also* brown tree snake
- bonobo (*Pan paniscus*) [375](#)
- Bornean orangutans (*Pongo pygmaeus wurmbii*) [374–375](#)
- BOTTLENECK [180](#), [495](#)
- bottlenecks [168–172](#)

- allelic diversity [172](#)
- definition [162](#), [168](#), [528](#)
- examples [115](#), [162–163](#), [169](#)
  - see also individual species*
- forensics *see* [forensics](#)
- inbreeding depression [301–302](#)
- microsatellites [170](#), [170](#)
- quantitative genetic diversity [172–173](#), [173](#)
- single pair [171](#)
- sustained vs. single generation [171](#)
- brain coral (*Goniastrea pauulus*) [276](#), [277](#)
- Branta sandvicensis* *see* [nene](#)
- breeding systems *see* [forensics](#)
- breeds, dogs [60](#), [61](#)
- British field cricket (*Gryllus campestris*) [321](#)
- brother–sister mating
  - inbreeding coefficient [263](#), [264](#)
  - inbreeding measurement [263](#)
- brown anole (*Anolis sagrei*) [418](#)
- brown bear (*Ursus arctos*) [148](#)
- brown rats (*Rattus norvegicus*) [425](#)
- Brown's banksia (*Banksia brownii*) [276](#)
- brown tree snake (*Boiga irregularis*) [414](#), [415](#), [469](#), [470](#)
- brush-tailed bettong (*Bettongia penicillata*) [352](#)
- Bt toxin transgenics, introgression [427](#)
- Bufo houstonensis*
  - see also* Houston toad
- Bufo marinus* *see* [cane toads](#)
- bullfrog (*Rana catesbeiana*) [352](#)
- bushtail possum (*Trichosurus vulpecula*) [414](#), [415](#), [421](#), [427](#)
- Cactoblastis moth (*Cactoblastis cactorum*) [421](#)
- Californian condor (*Gymnogyps californianus*)
  - bottlenecks [170](#)
  - captive breeding [435](#)
  - chondrodystrophy [121](#), [122](#), [122](#)

- allele frequencies [77](#), [130](#), [130](#), [267](#), [130](#)
- inbreeding [267](#)
- individual loci genetic management [226](#)
- management [449](#), [450](#)
- models [123](#)
- deleterious mutations [148](#)
- population targets [351](#)
- reintroduction [468](#), [468](#)
  - genetic diversity [462](#), [463](#)
- California redwood tree (*Sequoia senepervirens*) [371](#), [371](#)
- CAMPs (Conservation Assessment and Management Plans) [433](#)
- cane toads (*Bufo marinus*) [414](#), [419](#), [421](#)
  - biological control [421](#)
  - as invasive species [419](#), [419–420](#), [419](#)
- Canis latrans* (coyote), geographical genetic difference [332](#)
- Canis lupus* *see* [gray wolf \(\*Canis lupus\*\)](#)
- Canis lupus baileyi* *see* [Mexican wolf](#)
- Canis lupus rufus*
  - see also* [red wolf](#)
- Canis simensis* *see* [Ethiopian wolf](#)
- Canola (*Brassica napus*) [173](#), [326](#), [426](#)
- Cape Verde kite (*Milvus milvus*) [11](#)
- capped langur (*Trachypithecus pileatus*) [426](#)
- Capra* (ibex), outbreeding depression [381](#)
- Capricorn silvereye (*Zosterops lateralis chlorocephalus*) [503](#), [505](#)
- captive breeding programs [430](#)
  - extent of [431](#)
  - foundation [433](#), [434–437](#)
    - genetic backgrounds [437](#)
    - small founder numbers [435](#), [436–437](#)
    - source and numbers [434](#), [435](#)
    - unrelated founders [443](#)
    - wild-bred founders [434](#)
    - wild population numbers [435](#)
  - genetic adaptations to [458–461](#)
    - detection of [458](#)

- minimization of 458–459
- recovery from 461
- reintroduction problems 456–457, 458
- growth phase 371, 434, 437
  - target population size 437
- habitat loss 338
- heterozygosity 436, 436
- inbreeding depression 296
- IUCN 431
- maintenance phase 437–444
- reasons for 431–433
- stages in 433
  - see also specific stages*
- studbooks 432
- zoos 432–433
  - see also captive population management*
- captive population(s) 162
  - directional selection 134
  - founder effects 353
- captive population management 360, 437–444
  - current methods 439
  - effective population size 353, 353–354
  - environmental control 459
  - generation restriction 459
  - genetic deterioration 437–438
  - genetic diversity loss 438–439
  - genetic goals 351–355
    - generation length 351–355
  - groups 440, 444–446
    - adaptive genetic diversity 446
    - maximum avoidance of inbreeding 442, 444
    - non-threatened species 444
  - inbreeding 353, 438
    - avoidance 442–443
  - inbreeding depression 438
  - kinship minimization 443–444



- mean kinship minimization [439–441](#)
- mean kinship breeding strategies [441–442](#)
- pedigree information [443](#)
- population fragmentation [457](#), [459–461](#)
  - animal exchange [461](#)
  - experiments [460](#), [460](#)
- population sizes [438](#)
- preferential breeding control [459](#)
- reproductive fitness [351](#), [456](#), [457](#)
- CAPTURE [495](#)
- capture–mark–recapture, DNA-based censuring [478](#)
- CAPWIRE [495](#)
- CA repeats, microsatellite diversity [50](#), [58](#), [59](#)
- Caretta caretta* *see* [loggerhead turtle](#)
- Caribbean flamingo (*Phoenicopterus ruber*) [352](#)
- carp (European) (*Cyprinus carpio*) [424](#)
- carrier frequency, expected heterozygosity [77](#)
- Castanea dentata*
  - see also* American chestnut
- Castilleja vlinogosa* (giant red Indian paintbrush) [180](#)
- cat [210](#), [228](#), [393](#), [415](#), [447](#), [448](#)
- Catalania mahogany
  - introgression alleviation [427](#), [428](#)
  - wild population sizes [344](#)
- catastrophes
  - definition [528](#)
  - extinctions [7](#), [500](#)
  - population viability analysis [508](#), [509](#)
- cattle, inbreeding depression [286](#)
- cave bears [208](#)
- caviar, forensic detection [473](#)
- CBSG (Conservation Breeding Specialist Group) [433](#)
- cDNA [208](#)
  - definition [528](#)
- cDNA expression microarrays [210–211](#), [211](#)
  - definition [210](#), [211](#)

- hybrid vigour measurement 223
- transcription level measurement 210
- Center for Plant Conservation (USA) 431
- centromeres 213
- CERVUS 495
- Cervus eldii* 507
  - see also Eld's deer
- cetacea, molecular sexing 492
- chaffinch (*Fringilla coelebs*) 418
- chance effects, small populations 163, 163–173, 164
- Chatham Island black robin (*Petroica traversi*)
  - bottlenecks 170
  - population size increase 396
- checkerspot butterfly (*Euphydryas editha monoensis*) 118, 505, 505–506, 522
- cheetah (*Acinonyx jubatus*)
  - genetic drift 164
  - inbreeding depression detection 303
  - population bottlenecks 481
  - recovered populations, sensitivity analyses 510
- Chen caerulescens* see snow goose
- chickens 176, 195, 210, 212, 294, 306
  - inbreeding depression 287
- chimpanzee (*Pan troglodytes*)
  - D9S905 microsatellites 74, 74
  - parentage determination 489
- Chinese muntjac (*Muntiacus reevesi*)
  - allopatric speciation 373
  - Indian muntjac vs. 55
- Chinese tallow (*Sapium sebifera*) 418
- Chinook salmon (*Oncorhynchus tshawytscha*) 252, 514
  - $N_e/N$  ratios 243
  - population viability analysis 513
- CH inversion, *Drosophila* 197
- chi-square ( $\chi^2$ ) test 72

chloroplast DNA  
  definition [528](#)  
  heterozygosity loss in small populations [241](#)  
Choco palm (*Astrocaryum mexicanum*), extinction causes  
  demographic stochasticity [508](#)  
  environmental stochasticity [507](#), [508](#), [508](#)  
chondrodystrophy allele [77](#), [121](#), [122](#), [122](#), [123](#), [130](#), [130](#), [226](#), [264](#), [449](#),  
[450](#)  
chromosomes  
  allopatric speciation [374](#)  
  genetic diversity measurement [55](#)  
  mutations [142](#)  
*Chrysocyon brachyurus*  
  see also maned wolf  
chrytid fungus [29](#), [415](#)  
*Cicindela puritana*  
  see also puritan tiger beetle  
cis, definition [528](#)  
cis-regulatory regions [221](#)  
CITES see [Convention on International Trade in Endangered Species of Wild Flora and Fauna \(CITES\)](#)  
clades [528](#)  
*Clarkia pulchella*  
  see also evening primrose  
climate change see [global climate change](#)  
clines [155–158](#)  
  definition [153](#), [528](#)  
  environmental gradients vs. [158](#)  
  heavy-metal tolerance [155](#), [156](#)  
  migration–selection equilibria [158](#)  
  quantitative characters [156](#)  
  steepness [156](#)  
clonal tumours, Tasmanian devils [224](#)  
clones/cloning [448](#), [528](#)  
close relative mating exclusion, effective population size measurement  
[250](#)

- coadapted gene complexes, outbreeding depression [383](#)
- coalescence [254–256](#)
  - definition [255](#), [255](#), [528](#), [528](#)
  - forensics [476–477](#)
  - gene trees [476–477](#)
  - mitochondrial DNA data [477](#)
  - neutral theory of molecular evolution [255](#)
- coalescent theory [528](#)
- coancestry [528](#)
- co-dominance [44](#), [529](#)
- coefficient of linkage disequilibrium (*D*) [529](#)
- collecting, forensics [473](#), [473–474](#)
- colonial bentgrass (*Agrostis tenuis*) [155](#), [156](#), [197](#)
- COLONY [495](#)
- Columba mayeri* see [pink pigeon](#)
- combined probability method, meta-analysis [31](#)
- common ancestors [263](#), [529](#)
  - pedigrees [275](#), [275](#), [276](#)
- common cordgrass (*Spartina anglica*) [417](#), [418](#)
- common garden experiment
  - definition [529](#)
  - quantitative variation detection [94](#)
- complete dominance, selection [127](#)
- complete isolation see [population fragmentation](#)
- compound autosomal strains, invasive species control [423](#), [424–425](#)
- computer models see [models/simulations](#)
- coniferous New Zealand plant (*Halocharpus bidwilli*) [240](#)
- Conradina glabra*
  - see also *Apalachicola rosemary*
- conservation, biodiversity importance [2–3](#)
- Conservation Assessment and Management Plans (CAMPs) [433](#)
- Conservation Breeding Specialist Group (CBSG) [433](#)
- conservation genetics, definition [8–9](#)
- conspecific, definition [529](#)
- Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) [498](#)

- definition [528](#)
- convergent evolution [118](#)
  - Darwin's medium ground finch [118](#)
  - definition [529](#)
- Cooke's kok'io (*Kokia cookei*) [162](#), [431](#)
- copepods [382](#), [383](#)
- copy number variation
  - fitness [215](#)
  - genetic diversity [56](#)
  - genomes [214–215](#)
- correlation [102](#)
- corridors *see* [migration corridors](#)
- Corrigan grevillea (*Grevillea scapigera*)
  - translocation [403](#)
  - wild population sizes [344](#)
- cotton topped tamarins (*Saguinus oedipus*), heritability estimation [103](#)
- Coturnix japonica* (Japanese quail), inbreeding [22](#)
- covariance [101](#)
- cow [448](#)
- coyote (*Canis latrans*), geographical genetic difference [332](#)
- creeping bentgrass (*Agrostis stolonifera*) [426–427](#)
- critically endangered species [162](#)
  - definition [529](#)
  - IUCN definition [5](#), [5](#), [6](#)
- Crotalus durissus unicolor*
  - see also* Aruba island rattlesnake
- Cryan's buckmoth (*Hemileuca*), taxonomy [387](#)
- cryopreservation [447–448](#)
  - generation minimization [459](#)
  - plants [446](#)
- cryptic species [367](#)
  - heterozygote deficiency [79](#)
- Cumberlandian combshell mussel (*Epioblasma brevidens*) [466](#)
- Cunningham's skink (*Egernia cunninghami*), population fragmentation [333](#)
- curlew (*Numenius*), molecular sexing [492](#)

cyanogenic glucosides, as adaptive evolution [117](#)

*Cyprinodon alvarezi*

*see also* Potosi pupfish

cystic fibrosis [148](#)

cytochrome oxidase I locus, DNA barcoding [368](#)

*Danaus plexippus*

*see also* monarch butterfly

Darwin's finches (*Geospiza*), population bottlenecks [480](#)

Darwin's fox (*Lycalopex fulvipes*), wild population sizes [343](#)

Darwin's large cactus finch (*Geospiza conirostris*), reproductive success/family size [246](#), [246](#), [246](#)

Darwin's medium ground finch (*Geospiza fortis*)

convergent evolution [118](#)

reproductive success/family size [246](#)

selection response [105](#), [105](#), [106](#)

daughterless genes, invasive species control [425](#)

DDT usage, population size increase [396](#)

death adder (*Acanthophis* sp) [419](#)

deleterious alleles

equilibrium frequencies [177](#), [177](#)

evolutionary potential effects [60](#)

genetic diversity [60](#)

measurement [55](#)

inbreeding [55](#), [268](#)

inbreeding depression [297](#), [297–298](#)

individual loci genetic management [223](#)

mutation load [147](#), [148](#)

partially recessive [268](#)

population genomics [212](#)

removal of [176](#)

small populations [177](#), [177](#), [177](#)

variance [177](#)

deleterious mutations [146](#), [183](#)

accumulation [146](#), [147](#)

avoidance *see* [genetically viable populations](#)

- evolution [218–219](#), [220](#)
- genetically viable populations [342](#)
- inbreeding depression [300](#)
- mild [188](#)
- population genomics [212](#)
- removal [183](#), [188](#)
- deletions, genetic diversity [56](#)
- demographic history, forensics [478](#), [479](#)
- demographic stochasticity [499](#)
  - definition [529](#)
  - extinctions [7](#)
  - population viability analysis [507](#), [507](#), [508](#), [508](#)
- demography, definition [529](#)
- Diceros bicornis* *see* [black rhinoceros](#)
- Didelphis virginiana*
  - see also* opossum
- diet, forensics [493](#)
- dioecious, definition [529](#)
- Diplodus sargus*
  - see also* white sea bream
- Diornis*
  - see also* moa
- diploids
  - heterozygosity loss in small populations [241](#)
  - inbreeding depression [299](#)
  - mutation–selection balance [150](#)
- directional selection [104](#), [105](#)
  - allele frequencies [217](#)
  - captive populations [134](#)
  - definition [529](#)
  - evolution [216](#)
  - genetic diversity [134](#), [216](#)
  - gene trees [477](#), [477](#)
  - heavy-metal tolerance [134](#)
  - outbred populations [134](#)
  - quantitative characters [133](#), [133](#), [134](#)

- reproductive fitness [134](#)
- diseases
  - epidemiology [212](#)
  - forensics [493](#)
  - resistance [29–30](#)
  - see also* [pathogens](#)
- dispersal
  - forensics [484](#)
  - gene flow [328](#), [329–330](#)
  - outbreeding depression [382](#)
  - Y-specific markers [484](#)
- disruptive selection
  - definition [529](#)
  - heavy-metal tolerance [136](#)
  - quantitative characters [133](#), [136](#)
- DNA amplification, genetic diversity measurement [48](#), [49](#)
- DNA barcoding [368](#), [368–369](#)
  - controversy [369](#), [379](#)
  - definition [529](#)
  - plants [363](#)
- DNA-based censusing [478](#)
  - allelic dropout [478](#)
  - capture–mark–recapture [478](#)
  - errors [478](#)
  - rarefaction analysis [478](#)
- DNA fingerprints
  - definition [529](#)
  - genetic diversity measurement [51–52](#), [54](#)
- DNA samples, genetic diversity [48–54](#)
- DNA sequencing
  - genetic diversity [53](#), [54](#)
  - mitochondrial DNA [54](#)
  - species definition [367](#)
- DNA<sub>sp</sub> [204](#), [228](#)
- dogs, breeds [60](#), [61](#)
- dominance



- additive 127
- autosomal loci 128, 129
- complete, selection 127
- definition 529
- inbreeding depression 297–298
- partial see [partial dominance](#)
- selection 127, 127, 129, 130
- sex-linked loci 128, 129
- dominance variance ( $V_D$ ) 97, 98, 108–109
  - definition 529
  - estimation 108, 109
  - magnitude 109, 109
- dominant loci, genotypic variables 98, 98
- Dorcas gazelle (*Gazella dorcas*)
  - inbreeding coefficient 275, 276, 276
  - inbreeding depression 304
- DRIFT 180
- drift see [genetic drift](#)
- Drosophila*
  - alcohol dehydrogenase 57
    - balancing selection 192
    - coalescence/gene trees 254
  - bottleneck studies 170, 173
  - CH inversion, balancing selection 197
  - coding loci numbers 213
  - directional selection 135
    - effective population size vs. 346
  - evolutionary potential studies 238
  - homozygosity 152
  - inbreeding depression 341
  - population fragmentation 318, 318, 460
  - random genetic drift 321
  - recessive lethal models 124, 124
  - seasonal chromosome inversions 197
- Duchenne's muscular dystrophy 148

Durrell, Gerald [432](#)  
dusky seaside sparrow [364](#), [486](#), [500](#)

Eastern barred bandicoot (*Perameles gunni*) [343](#)  
    reproductive success/family size [246](#)  
    wild population sizes [343](#)

EASYPOP [159](#), [180](#), [204](#), [257](#), [335](#)  
ecological exchangeability, taxonomy [385](#)  
ecosystem disturbance, invasive species [416](#)  
ecosystem services [2](#)  
ecotypes [117](#), [529](#)  
effectively neutral alleles [199](#), [529](#)  
effective number of alleles ( $n_c$ ) [74](#), [530](#)  
effective population size ( $N_e$ ) [242–243](#), [500](#)  
    captive population management [353](#), [353–354](#)  
    definition [176](#), [530](#)  
    estimation [252–254](#), [253](#), [254](#)  
        drift–mutation equilibrium [253](#), [254](#)  
    measurement [243–254](#)  
        close relative mating exclusion [250](#)  
        combined factors [251](#), [251–252](#)  
        family size variation [245–247](#), [248](#)  
        inbreeding [252](#)  
        overlapping generations [251](#)  
        population size fluctuations [244](#), [244](#), [245](#)  
        unequal sex-ratio [249–250](#)  
     $N_e/N$  ratios [242–243](#)  
        fecundity [243](#)  
    real vs. [176](#)  
    small populations [176](#)

effect size analysis, meta-analysis [32](#)

*Egernia cunninghami*  
    see also Cunningham's skink

eider duck (*Somateria mollissima*), genotype frequencies [67](#), [68](#), [71](#)

*Elaphurus davidianus*

- see also* Père David deer
- Eld's deer (*Cervus eldii*), inbreeding depression 507, 507
- electrophoresis
  - definition 530
  - protein separation 46
- Elephas maximus* *see* Asian elephant
- endangered species 3–5, 20–22, 162
  - definitions 5–6, 530
    - IUCN definition 5, 5, 6
  - evolutionary potential 176
  - extent of 4
  - genetically viable populations 341
  - genetic diversity loss 240, 240, 246
  - heritability 108
  - listing importance 6
  - see also individual species*
- endangerment
  - causes 498–500, 515
  - deterministic factors 499
    - stochastic factor interactions 500
  - stochastic factors 499–500
    - deterministic factor interactions 500
- endemic 530
- Enhydra lutris*
  - see also* sea otter
- environment
  - control, captive population management 459
  - genetically viable populations 343
  - genetic diversity 310
  - gradients vs. clines 158
  - outbreeding depression effects 381
  - speciation 370
- environmental agents, mutations 142
- environmental stochasticity 499
  - definition 530
  - extinctions 7

- population viability analysis [508](#), [508](#)
- environmental stress, inbreeding depression [294](#), [294](#)
- environment variation ( $V_E$ ) [96](#)
  - partitioning [91–92](#), [96](#), [96–97](#)
  - quantitative variation [93](#)
  - Seychelles warbler [93](#)
- Epilobium angustifolium* (Pireweed) [299](#)
- Epioblasma brevidens*
  - see also* Cumberlandian combshell mussel
- epistasis [530](#)
- epistatic variance [530](#)
- equalization of family size (EFS) [247](#)
- equilibrium [69](#), [530](#)
- equilibrium frequencies [149](#), [150](#)
  - deleterious alleles *see* [deleterious alleles](#)
  - heterozygote advantage [190](#), [191](#)
  - sex-linked loci [149](#)
  - small population genetic diversity maintenance [199](#)
  - tetraploids [150](#), [151](#)
- Equus ferus przewalskii*
  - see also* Przewalski's horse
- Equus grevyi*
  - see also* Grevy's zebra
- Eschrichtius robustus*
  - see also* gray whale
- establishment, invasive species [416](#)
- ethics, conservation [3](#)
- Ethiopian wolf (*Canis simensis*)
  - allele diversity [74](#), [74–75](#)
  - introgression [426](#)
  - microsatellites [154–155](#)
  - wild population sizes [343](#)
- Eubalaena glacialis*
  - see also* Northern Atlantic right whale
- eucalypt trees

- dispersal distances [330](#)
- glaucousness, balancing selection [197](#), [197](#)
- Euphydryas editha bayensis*
  - see also Bay checkerspot butterflies
- Eurasian cheat grass (*Bromus tectorum*) [415](#)
- European bison (*Bison bonasus*)
  - bottlenecks [169](#)
  - captive breeding, founder numbers [435](#)
- evening primrose (*Clarkia pulchella*), inbreeding [24](#)
- evolution
  - adaptive see [adaptive evolution](#)
  - convergent see [convergent evolution](#)
  - definition [530](#)
  - effective population size < 500 [345](#), [346](#)
  - genetic diversity [29](#)
  - migration [120](#)
  - mutations [120](#)
  - necessity of [116–119](#)
  - population genomics [216–222](#)
    - deleterious mutations [218–219](#), [220](#)
    - directional selection [216](#)
    - mutation sites [221–222](#)
    - non-coding DNA selection [220–221](#)
    - synonymous variants [221](#)
  - reproductive fitness effects [345](#), [346–347](#)
  - species definition [367](#)
- evolutionary potential [9](#), [95](#)
  - definition [530](#)
  - deleterious alleles [60](#)
  - endangered species [176](#)
  - genetically viable populations [337](#)
  - population genomics [212](#)
  - threatened populations [345–347](#)
- evolutionarily significant units (ESUs) [384–385](#)
  - definition [530](#)
- exchangeability, species management units [385–387](#)

exons

definition [530](#)

genetic variability [56](#)

expected heterozygosity ( $H_e$ ) [69](#), [72–78](#), [84](#)

carrier frequency [77](#)

definition [530](#)

expected DNA sequence variation [76](#)

Laysan finch microsatellites [72](#), [80](#), [84](#)

nucleotide diversity [76](#)

*see also* [allele diversity \(A\)](#)

*ex situ* conservation [360](#), [431](#)

definition [530](#)

plants [446](#)

*see also* [captive breeding programs](#)

extinction [19–33](#)

catastrophes [7](#), [500](#)

causes [7](#), [498–500](#)

human-associated factors [7](#)

*see also* [specific causes](#)

definition [530](#)

genetic factors [7](#)

genetic diversity loss vs. [28–30](#)

haplo-diploid species [410](#), [410–411](#)

islands [3](#)

population re-establishment [405](#)

population viability analysis [506–509](#)

catastrophes [508](#), [509](#)

demographic stochasticity [507](#), [507](#), [508](#), [508](#)

environmental stochasticity [508](#), [508](#)

genetics [506](#)

immigration [509](#)

inbreeding depression [506–507](#), [507](#)

projected rates [4–5](#)

recorded [3](#), [3–4](#)

species regeneration [448–449](#)

VORTEX [34](#)

‘extinction vortex’ [7](#), [24](#), [24–25](#), [530](#)

extra-pair copulation [490](#)

*F*, definition [530](#)

*Falco peregrinus*

see also peregrine falcon

*Falco punctatus* see [Mauritius kestrel](#)

family size

definition [245](#)

effective population size measurement [245–247](#), [248](#)

population viability analysis [506–507](#)

fecundity,  $N_e/N$  ratios [243](#)

fin whales (*Balaenoptera physalus*), effective population size estimation  
[254](#)

fire ant (*Solenopsis* sp.) [415](#), [419](#)

fireweed (*Epilobium angustifolium*) [299](#)

$F_{IS}$ , definition [530](#)

fish

selective harvesting [406](#)

transgenics, introgression [427](#)

Fisher's combined probability test [32](#)

fish hatcheries, supportive breeding [465](#)

$F_{IT}$  [530](#)

fitness see [reproductive fitness](#)

fixation [128](#), [164](#), [168](#)

definition [530](#)

fixation index ( $F_{ST}$ ) [328](#)

Florida panther (*Puma concolor*)

deleterious mutations [148](#)

genetic issues [392–394](#)

introgression [426](#)

migration corridors [392–394](#), [402](#)

wild population sizes [343](#)

fluctuating populations, inbreeding [272](#)

- forensics [9](#), [13](#), [360](#), [472–481](#)
  - aging/fitness [493](#)
  - bottlenecks [479–481](#)
    - microsatellites [479](#)
  - breeding systems [486–488](#)
    - biparental inbreeding [488](#)
    - multilocus data [488](#)
    - selfing [487](#), [488](#)
  - definition [530](#)
  - demographic history [478](#), [479](#)
  - diet [493](#)
  - diseases [493](#)
  - founder relationships [490](#)
  - founder sourcing [491](#), [491](#)
  - gene flow [481–486](#)
    - dispersal [484](#)
    - immigrant detection [485](#)
    - phylogeography [485–486](#)
    - population structure [484](#)
    - secondary contact [481](#)
    - sub-species rescue [485](#)
  - illegal hunting/collecting [473](#), [473–474](#)
  - molecular clock dating [494](#)
  - parentage [489–490](#)
    - extra-pair copulation [490](#)
  - pedigree reconstruction [490](#)
  - population size [478](#)
  - reintroduction [475](#), [486](#)
  - sexing [491–493](#)
  - species biology [474–477](#)
    - coalescence [476–477](#)
  - telomere lengths [493](#)
  - translocation [486](#)
- foundation, captive breeding programs *see* [captive breeding programs](#)
- founders
  - captive populations [353](#)



- numbers 435, 436–437
- definition 531
- inbreeding in small random mating populations 269
- invasive species 418
- relationships
  - forensics 490
  - population genomics 212
- sourcing 491, 491
- wild-bred, captive breeding programs 434
- fox, red (*Vulpes vulpes*) 139, 356
- fragmented population(s)
  - habitat fragmentation 399, 400
  - Hardy–Weinberg equilibrium 79
  - heterozygote deficiency 79
  - homozygosity 79
  - resolution of 10
- fragmented population management 399
  - extinct population re-establishment 405
  - gene flow management 401, 402–403
  - migration corridors see migration corridors
  - translocation 400
    - definition 538
    - drawbacks 400
    - forensics 486
    - genetic issues 403
- Frankel, Otto 7
- $F_{ST}$ , 312, 321, 322, 323, 324, 324, 326 531
  - gene flow inferred from 327, 328, 330, 331
  - population structure and 483
  - taxa 328
- FSTAT 87, 335
- $F$  statistics 531
  - multigeneration effects 323
- full-sib correlations, heritability estimation 103
- full-sib mating

- definition [531](#)
- inbreeding increase [276](#), [277](#), [278](#)
- recurrence relationships [277](#)
- functional DNA variability [57](#)
- functional genomics, cDNA expression microarrays [210](#)
- functional RNA, genome [213](#)
- Furbish's lousewort (*Pedicularis furbishiae*), population viability analysis [513](#)

*Gadus morhua*

*see also* Atlantic cod

Galápagos tortoises

forensic biology [474](#)

population bottlenecks [479](#)

*Gallirallus owstoni* *see* [Guam rail](#)

*Gallirallus sylvestris* *see* [Lord Howe Island woodhen](#)

GARFIELD [228](#)

*Gasterosteus aculeatus*

*see also* three-spined stickleback fish

*Gazella dorcas* *see* [Dorcas gazelle](#)

*Gazella spekei*

*see also* Speke's gazelle

GDA [87](#)

GEMINI [64](#)

GENALEX [87](#)

GenAlEx [335](#)

gene(s), definition [43](#)

GENECLASS2 [335](#)

gene diversity *see* [expected heterozygosity \( \$H\_e\$ \)](#)

gene drop [531](#)

gene expression studies [222–223](#)

gene flow

definition [531](#)

forensics *see* [forensics](#)

management

computer models [401](#), [403](#)

- fragmented population management [401](#), [402–403](#)
- migrants [325](#)
- multilocus microsatellites [328](#)
- population fragmentation [325–327](#)
  - dispersal [328](#), [329–330](#)
  - distance between fragments [330](#), [331](#)
  - inbreeding [326](#)
  - island populations [325](#), [325](#)
  - measurement [327–331](#)
  - migration [326](#), [326](#)
  - non-idealized populations [327](#)
  - restrictions [327](#)
- gene genealogies [531](#)
- GENEPOP [335](#)
- generation length
  - captive population management [351–355](#)
  - genetic diversity loss [237](#)
- generation number restriction, captive population management [459](#)
- genetic(s) [7](#), [8](#)
  - extinctions [7](#)
  - invasive species [418](#), [418](#)
  - population viability analysis *see* [population viability analysis \(PVA\)](#)
  - pre-extinction effects [27–28](#)
  - use examples [10–13](#)
- genetically viable populations [337](#)
  - deleterious mutation avoidance [348–349](#)
    - experimental evidence [349](#)
  - evolutionary potential [340](#), [341–343](#)
    - deleterious mutations [342](#)
    - environmental effects [343](#)
    - genetic variation [342](#)
    - mutation [342](#)
    - reproductive fitness [343](#)
  - genetic diversity regeneration [340](#), [348](#)
  - reproductive fitness [340–341](#)
  - single locus genetic diversity [347](#)

- size 340
- small population fallacy 355–356
- space constraints 338, 338–340
- threatened populations 343–344, 343–347
  - effective population size <500 345
  - evolutionary potential 345–347
- see also captive population management; wild population management
- genetic backgrounds, captive breeding program foundation 437
- genetic barriers, population fragmentation 310
- genetic control, invasive species control 423–425
- genetic deterioration, captive population management 437–438
- genetic distance 44, 531
- genetic diversity 9, 41–45
  - allele frequencies 67, 67–69, 68
  - allelic diversity 43
  - changes in 40
  - conservation of 42
  - copy number variation 56
  - definition 7, 22, 43, 86, 531
  - deletions 56
  - directional selection 134
  - disease resistance 29–30
  - environment effects 310
  - evolution 29, 42
  - extent 39, 56–61
    - deleterious alleles 60
    - microsatellites 58–59
    - nuclear DNA 56–57
    - protein variation 45, 59, 59
    - quantitative variation 60–61
- GEMINI 64
- genotype frequencies 67–69
- heterozygosity 43
- importance of 42–43
- inbreeding vs. 282

- inversions [56](#)
- loss *see below*
- maintenance *see below*
- measurement [46–56](#), [67](#)
  - allozymes [54](#)
  - amplified fragment length polymorphisms [50–51](#), [54](#)
  - chromosomes [55](#)
  - deleterious alleles [55](#)
  - DNA amplification [48](#), [49](#)
  - DNA fingerprints [51–52](#), [54](#)
  - DNA samples [48–54](#), [50](#)
  - DNA sequencing [53](#), [54](#)
  - microsatellites [49](#), [50](#), [54](#), [65](#)
  - mitochondrial DNA [50](#), [54–55](#)
  - mode of inheritance [54](#)
  - PCR [48](#), [49](#)
  - proteins [46–48](#)
  - qualitative characteristics [55](#)
  - randomly amplified polymorphic DNA [51](#), [54](#)
  - restriction fragment length polymorphisms [52–53](#)
  - single-nucleotide polymorphisms [51](#), [54](#)
  - single-strand conformational polymorphisms [53](#), [54](#)
- mutations/migrations [141](#)
- neutral theory of molecular genetics [186](#)
  - observed levels vs. [187](#), [187](#)
- origin/regeneration [141](#)
- plant self-incompatibility loss [29](#)
- polymorphisms [43](#)
- population size vs. [239](#), [240](#)
  - within species [240](#)
- quantitative variation *see* [quantitative variation](#)
- regeneration [340](#), [348](#)
- regeneration times [144–145](#), [146](#)
- reintroduction [462](#), [463](#)
- reproductive fitness vs. [42–43](#)
- self-fertilizing species [408](#)

- single loci *see* [single loci variation](#)
- species-dependent [63](#), [63](#)
- terminology [44](#)
- variations in [62–63](#)
  - global climate change [63](#)
  - $G_{ST}$  values [59](#), [62](#)
  - large populations [62](#)
  - plants [62](#)
  - small populations [63](#)
- wild population management [394](#)
- genetic diversity loss [27](#), [30–33](#)
  - captive population management [438–439](#)
  - directional selection [216](#)
  - extinction vs. [28–30](#)
  - fitness vs. [232–234](#), [233](#)
  - over time [232](#)
  - population size reduction [229–230](#)
  - sustained population size restrictions [234–241](#), [235](#), [237](#)
    - endangered species [240](#), [240](#), [246](#)
    - generation length effects [237](#)
    - haploid loci [241](#)
    - heterozygosity reduction [236](#), [236](#), [237](#), [237](#), [239](#)
    - microsatellite variation [237](#), [238](#)
    - neutral loci [237](#), [238](#)
    - polyploid loci [241](#), [242](#)
    - population fluctuation [238](#)
    - sex-linked loci [241](#)
    - wild populations [222–223](#), [240](#)
- threatened species [61](#), [62](#)
- genetic diversity maintenance
  - balancing selection *see* [balancing selection](#)
  - large populations [184](#)
  - large vs. small populations [183](#)
  - mutation–selection balance [146](#)
  - neutral mutations under random genetic drift [184–188](#), [185](#)

- amino acid substitutions 186
- non-translated DNA 186
- synonymous mutations 186
- selection intensity variation 188–189, 201
- small populations 199–204
  - associative overdominance 202–204, 203
  - balancing selection 199
  - equilibrium frequency 199
  - genetic drift 199, 199, 199–200, 200, 201
  - large populations vs. 183
  - mutation–selection equilibria 199
  - selection 199, 199–200, 200, 201
  - selection vs. drift 201–202
- see also* mutation(s)
- genetic drift 9
  - allele frequency models 165–168, 166
  - allele loss 30, 177
  - chance effects 164–165
  - definition 28, 163, 531
  - genetic diversity maintenance 199, 199, 199–200, 200, 201
  - models 164, 165
  - mutation equilibrium, effective population size estimation 253, 254
  - neutral mutations 184
  - random diversification 164
  - small populations 30, 177, 199, 199, 199–200, 200, 201
  - see also* fixation
- genetic erosion 531
- genetic exchangeability, taxonomy 385
- genetic load 531
- genetic management
  - individual loci 223–226
  - implementation 226
  - population genomics 212
  - reintroduction 461–464
  - threatened species 4, 13–14
- genetic markers

- allopatric speciation *see* [allopatric speciation](#)
- inbreeding depression [303](#)
- polymorphic, population structure [482](#)
- genetic rescue [22](#), [398](#)
  - definition [20](#), [397](#), [531](#)
  - inbreeding depression [288–289](#), [306–307](#)
  - single populations [399](#)
  - unrelated individual sources [397](#), [398](#)
    - sub-species [398](#)
- genetic stochasticity [499](#)
  - definition [531](#)
- genetic variability
  - exons [56](#)
  - genetically viable populations [342](#)
  - introns [56](#)
  - invasive species [418](#), [418](#)
- genetic variance, additive [97](#), [98](#), [99](#)
- gene trees [254–256](#)
  - coalescence [476–477](#)
  - definition [531](#)
  - directional selection [477](#), [477](#)
  - migration [477](#), [477](#)
  - selective sweeps [477](#)
- genome(s) , [212–216](#)
  - coding loci [213](#)
    - taxonomic differences [213–214](#)
  - complexity origins [216](#)
  - components [213](#)
    - see also specific components*
  - copy number variation [214–215](#)
  - definition [44](#), [531](#)
  - mammals [208](#)
  - sequencing *see below*
  - transposable elements [215](#)
    - see also specific types*
- genome-enabled taxa [208](#), [531](#)



genome resource banks [447–449](#)  
definition [531](#)  
genome sequencing [208–210](#)  
benefits from [211](#)  
costs [208](#)  
process [208](#)  
species numbers [209](#), [212–216](#)  
threatened species [208](#)  
genome × environment interaction  
definition [531](#)  
quantitative variation [110–111](#)  
genotype(s), definition [44](#)  
genotype frequencies  
genetic diversity [67–69](#)  
Hardy–Weinberg equilibrium *see* [Hardy–Weinberg equilibrium](#)  
inbreeding [266](#)  
population fragmentation [320](#)  
genotypic variables, additive loci [98](#), [98](#)  
GENSTAT [112](#)  
*Geochelone peltastes*  
*see also* Rodriguez giant tortoise  
*Geochelone vosmaeri*  
*see also* Rodriguez giant tortoise  
*Geospiza*  
*see also* Darwin's finches  
*Geospiza conirostris*  
*see also* Darwin's large cactus finch  
*Geospiza fortis* *see* [Darwin's medium ground finch](#)  
*Geronticus eremita*  
*see also* Waldrapp ibis  
*Geum radiatum*  
*see also* spreading avens plant  
ghost bat (*Macroderma gigas*), population structure [483](#)  
giant frog (*Cyclorana australis*) [419](#), [420](#)  
giant panda (*Ailuropoda melanoleuca*)  
habitat fragmentation [399](#), [410](#)

- wild population sizes [343](#)
- giant red Indian paintbrush (*Castilleja vlinogosa*) [180](#)
- GIMLET [495](#)
- Glanville fritillary (*Melitaea cinxia*)
  - genetic diversity loss [234](#)
  - metapopulations [313](#)
- Glaucopsyche lygdamus palosverdesensis*
  - see also* Palo Verdes blue butterfly
- global climate change [xvii](#), [28](#), [158](#), [229](#), [518](#)
  - evolution necessity [116](#), [119](#)
  - genetic diversity [63](#)
  - projected extinction rates [5](#)
  - translocation [403](#)
- global trade, invasive species [415](#)
- Globicephala melas*
  - see also* long-finned pilot whale
- Gloger's rule [155](#)
- glucose-6-phosphate dehydrogenase deficiency [182](#)
  - heterozygote advantage [192](#)
- glyphosate resistance, introgression [426](#)
- goanna (*Varanus* sp) [419](#), [420](#)
- golden-headed lion tamarin (*Leontopithecus chrysomelas*), reproductive success/family size [246](#)
- golden langur (*Trachypithecus geei*) [593](#)
- golden lion tamarin (*Leontopithecus rosalia*)
  - captive breeding programs
    - effective population size [353](#), [353–354](#)
    - founder numbers [435](#)
    - mean kinship values [441](#)
    - population growth [371](#)
    - wild-bred founders [434](#)
  - deleterious mutations [148](#)
  - effective population size measurement [251](#), [251–252](#)
  - inbreeding depression [287](#), [288](#)
  - migration corridors [400](#), [402](#)
  - minimum habitat area [519](#)

- population viability analysis [501](#), [502](#)
- reintroductions [454](#), [454–456](#)
- reproductive success/family size [246](#)
- wild population management [349](#), [350](#)
- wild population sizes [344](#)
- Goniastrea favvulus*
  - see also* brain coral
- grassland daisy (*Rutidosia leptorrhynchoides*) [29](#)
  - genetic diversity loss vs. reproductive fitness [233](#)
- gray whale (*Eschrichtius robustus*), effective population size estimation [254](#)
- gray wolf (*Canis lupus*)
  - deleterious mutations [148](#)
  - evolutionary potential [345](#), [346–347](#)
  - inbreeding coefficient estimates [272](#), [273](#), [273–274](#)
  - isolation by distance [331](#), [331](#)
- greater glider (*Petauroides volans*), population fragmentation [330](#)
- greater prairie chicken (*Tympanuchus cupido*)
  - extinction vortex [500](#)
  - genetic diversity loss [235](#)
  - genetic rescue [306](#), [306](#)
  - inbreeding depression [306](#), [306](#)
  - population bottlenecks [479](#)
- Grevillea scapigera* *see* [Corrigan grevillea](#)
- Grevy's zebra (*Equus grevyi*), reproductive success/family size [246](#)
- groups, captive population management *see* [captive population management](#)
- growth phase, captive breeding programs *see* [captive breeding programs](#)
- Grus americana* *see* [whooping crane](#)
- Grus japonensis*
  - see also* red-crowned crane
- Grus vipio*
  - see also* white-naped crane
- Gryllus campestris*
  - see also* British field cricket

$G_{ST}$  values, genetic diversity [59](#), [62](#)  
Guam rail (*Gallirallus owstoni*)  
    bottlenecks [170](#)  
    captive breeding, founder numbers [435](#)  
    reintroduction [469](#)  
*Gymnogyps californianus* see [Californian condor](#)  
gymnosperms, inbreeding depression [296](#)  
gynodioecious plants  
    definition [531](#)  
    wild population management [409](#)  
gypsy moth (*Lymantria dispar*) [415](#)  
  
H5N1 influenza [29](#)  
habitat fragmentation [310](#)  
    fragmented populations [399](#), [400](#)  
    ‘island’ patches [310](#)  
    multigeneration effects [311–312](#), [315](#)  
    population fragmentation [230](#)  
habitat loss [338](#)  
haemophilia  
    frequency [148](#)  
    mutation rate estimation [151](#), [151](#)  
half-sib correlations, heritability estimation [103](#)  
half-sib mating [274](#), [274](#), [281](#)  
*Haliaeetus leucocephalus*  
    see also bald eagle  
*Halcyon cinnamomina cinnamomina*  
    see also Micronesian kingfishers  
*Haloragodendron lucasii* [487](#)  
haplo-diploid species  
    extinction [410](#), [410–411](#)  
    inbreeding depression [410](#)  
    wild population management [409–410](#)  
haploids  
    genetic diversity loss [241](#)  
    heterozygosity loss in small populations [241](#)

- inbreeding depression 299
- mutation–selection balance 150
- wild population management 412
- haplotype 531
- haplotype diversity ( $h$ )
  - definition 532
  - expected heterozygosity 76
- haplotype networks
  - definition 532
  - population structure 482, 483
- Hardy–Weinberg equilibrium 68, 69–72
  - allele vs. genotype frequencies 70
  - definition 69, 532
  - deviations from 78–79
    - fragmented populations 79
    - inbreeding 78, 78, 79
  - expected heterozygosity see expected heterozygosity ( $H_e$ )
  - extensions of 79–83
    - polyploids 82–83
    - sex-linked loci 72, 79, 80–82
    - three alleles 70, 79–80, 80
  - genotype frequencies 69, 70, 71
    - allele vs. 70, 71
  - hermaphroditic marine organism model 69, 70
  - outbreeding populations 71
- harmonic mean
  - definition 532
  - population sizes 244
- harvesting impacts, wild population management 406, 406–407
- Hawaiian goose see nene (*Branta sandvicensis*)
- Hawaiian Laysan finch see Laysan finch (*Telespiza cantans*)
- heavy-metal tolerance
  - balancing selection 197
  - clines 155, 156
  - directional selection 134

- disruptive selection [136](#)
- evolution of [118](#)
- plants [42](#)
- Heliconius*, sex-linked 6-phosphogluconate dehydrogenase [81](#)
- Helonias bullata*
  - see also Swamp pink
- Hemileuca*
  - see also Cryan's buckmoth
- herbicide resistance, introgression [426](#)
- heritability ( $h^2$ ) [95](#)
  - definition [100](#), [532](#)
  - in endangered species [108](#)
  - estimation [100–103](#)
    - full-sib correlations [103](#)
    - half-sib correlations [103](#)
  - magnitudes of [105–107](#)
  - quantitative characters [94](#)
- hermaphrodites
  - definition [532](#)
  - Hardy–Weinberg equilibrium model [69](#), [70](#)
- Heterocephalus glaber*
  - see also naked mole rat
- heterogenous environments, balancing selection [197](#)
- heterosis see [hybrid vigour \(heterosis\)](#)
- heterozygote advantage (overdominance) [184](#)
  - balancing selection [189–192](#)
  - definition [532](#), [534](#)
  - equilibrium frequencies [190](#), [191](#)
  - small populations [201](#), [202](#)
- heterozygotes/heterozygosity
  - average see [average heterozygosity \( \$H\$ \)](#)
  - bottlenecks [170](#)
  - captive breeding programs [436](#), [436](#)
  - deficiency
    - cryptic species [79](#)

- fragmented populations 79
- definition 44, 532
- expected see expected heterozygosity ( $H_e$ )
- expression of 73
- fitness vs. 232, 234
- genetic diversity 43
- inbreeding vs. 266, 282, 282
- loss of 86
  - genetic diversity loss 236, 236, 237, 237, 239
- major histocompatibility complex polymorphism 194
- multigeneration effects 319, 320
- population fragmentation 319
- self-fertilizing species 408
- Hexaprotodon liberiensis* 263
  - see also pigmy hippopotamus
- HIV, major histocompatibility complex polymorphisms 30
- homoplasy 532
- homozygotes/homozygosity
  - definition 44
  - inbreeding 265, 266, 267
  - recessive 267
- honey suckle (*Lonicera* sp) 420, 489
- Hong Kong lady's tresses orchid (*Spiranthes hongkongensis*), speciation 371, 372
- horse (*Equus*) 262, 468
  - Assateague Island horses 421, 422
  - molar height, long-term adaptive changes 135, 135
  - see also Przewalski's horse
- Houston toad (*Bufo houstonensis*), heterozygosity vs. generation time 352
- human factors
  - extinctions 7
  - island extinctions 26
- humans
  - coding loci numbers 209, 213

- genome deletion variants [215](#)
  - inbreeding depression [286](#)
  - stabilizing selection [136](#)
- humpback whale (*Megaptera novaeangliae*)
  - effective population size estimation [254](#)
  - wild population sizes [344](#)
- hunting
  - forensics [473](#), [473–474](#)
  - sex-ratio distortion [250](#)
- Huntington's chorea [148](#)
- hybridization
  - detection [12](#)
  - invasive species [417](#)
  - taxonomy problems [365](#)
- HYBRIDLAB [429](#)
- hybrid vigour (heterosis) [222–223](#)
  - definition [91](#), [223](#), [303](#), [532](#)
  - measurement, cDNA expression microarrays [223](#)
- Hymenoptera [409](#)
- Hymenoxys acaulis* var *glabra* see [Lakeside daisy](#)
- ibex (*Capra*), outbreeding depression [381](#)
- idealized populations
  - definition [532](#)
  - population fragmentation [316](#), [316](#)
  - small populations [174](#), [175](#), [175](#)
- identity by descent, definition [532](#)
- illegal hunting, forensics [473](#), [473–474](#)
- immigrants/immigration
  - forensics [485](#)
  - population viability analysis [509](#)
- immunocontraception, invasive species control [421–422](#), [422](#)
- impala (*Aepyceros melampus*), mitochondrial DNA haplotype networks [483](#)
- inbreeding [9](#), [20](#), [173](#)
  - allele frequencies [267](#)



- backcrossing *see* [backcrossing](#)
- captive breeding programs *see* [captive population management](#)
- computer models [20](#)
- conservation concerns [262](#), [263](#)
- definition [261](#), [532](#)
- effective population size measurement [252](#)
- extinction [7](#), [20](#), [22–28](#)
  - computer models [27](#)
  - wild populations [23](#), [23–24](#), [24](#)
- gene flow [326](#)
- genetic consequences [265–269](#)
  - deleterious alleles [55](#)
  - deleterious partially recessive alleles [268](#)
  - genotype frequencies [266](#)
  - heterozygosity [266](#)
  - homozygosity [265](#), [266](#)
  - lethal homozygotes [268](#), [268–269](#)
  - recessive homozygotes [267](#)
- genetic diversity *vs.* [282](#)
- Hardy–Weinberg equilibrium [78](#), [78](#), [79](#)
- heterozygosity *vs.* [282](#), [282](#)
- inbreeding depression *vs.* [301](#), [301](#)
- measurement [21–22](#), [262–265](#)
  - base population [265](#)
  - inbreeding coefficient *see* [inbreeding coefficient \( \$F\$ \)](#)
- minimization of [10](#)
- mutation–selection balance [279](#), [280](#)
- pedigrees [274–276](#)
  - common ancestors [275](#), [275](#), [276](#)
  - half-sib mating [274](#), [274](#)
- polypoids [281–282](#)
- population size *vs.* [230](#), [282](#)
- quantitative variation detection [94](#), [96](#), [100–102](#)
- regular systems [276–278](#)
  - recurrence relationships [277](#)
  - see also specific systems*

- reproductive fitness [262](#)
- small random mating populations [269–274](#)
  - accumulation of [271](#), [272](#)
  - founding individuals [269](#)
  - rate vs. population size [270](#)
  - theory of [269–272](#)
- wild populations [20](#)
- inbreeding coefficient ( $F$ ) [21](#), [262–265](#)
  - brother–sister mating [263](#), [264](#)
  - definition [263](#), [532](#)
  - half-sib mating [274](#), [281](#)
  - inbreeding depression vs. [298–299](#)
  - indirect estimates [272](#), [273–274](#)
  - reproductive fitness [340](#)
  - self-fertilization [263](#)
- inbreeding depression [221](#)
  - bottleneck effects [301–302](#)
  - captive population management [438](#)
  - characteristics [293–296](#)
  - definition [20](#), [532](#)
  - detection [288–289](#), [303–305](#), [306](#)
  - distribution [34](#)
  - environmental stress [294](#), [294](#)
  - extinctions [7](#), [290–293](#)
    - experiments [290](#)
    - progeny numbers [290](#)
  - genetic basis [296–299](#)
    - deleterious alleles [297](#), [297–298](#)
    - dominance [297–298](#)
    - multiple loci [298](#)
    - ploidy [299](#)
  - genetic rescue [288–289](#), [306–307](#)
  - haplo-diploid species [410](#)
  - historically small populations [302–303](#)
  - inbreeding coefficient vs. [298–299](#)
  - inbreeding rate vs. [301](#), [301](#)

- lethal equivalents 304–305, 306
  - definition 304
  - measurement 286–287, 303–304, 304
  - migration 141
  - models 296, 297
  - naturally outbreeding species 286–287
    - in wild 287, 288–289
  - plants 23
  - population viability 291–293
    - population growth rates 291
    - species 293
  - population viability analysis 505, 506, 506–507, 507
  - purging see purging
  - regularly inbred species 296
  - reproductive fitness 286–287, 293, 340
  - selection effects 300–301
    - deleterious alleles 300
  - small populations 289, 290
  - variability in 295–296
    - captivity effects 296
    - species variation 295
    - taxa specificity 296
  - wild vs. captive populations 296, 305
- indels 209, 221
  - definition 532
  - genetic diversity measurement 209
- Indian muntjac (*Muntiacus muntjak*)
  - allopatric speciation 373
  - Chinese muntjac vs. 55
- Indian rhinoceros (*Rhinoceros unicornis*) 351
  - bottlenecks 169
  - captive breeding, founder numbers 435
  - genetically viable populations 338
  - genetic viability 338
  - heterozygosity vs. generation time 352
  - population size increase 395

- individual choice, reintroduction [463–464](#)
- industrial melanism [115](#), [118](#)
  - adaptive evolutionary change [124](#)
  - balancing selection [197](#)
- information sources [15–16](#)
- inherited diseases
  - management [449–450](#), [450](#)
  - population genomics [211](#), [212](#)
- initial generation effects, population fragmentation [316–317](#), [317](#), [320](#)
- in situ* conservation [408](#), [532](#)
- interaction variance ( $V_I$ ) [97](#), [109](#)
  - definition [532](#)
- interfering RNA (RNAi) genes [214](#), [536](#)
- intergenic DNA [213](#)
  - selection [220](#)
- introduced species *see* [invasive species](#)
- introgression [532](#)
  - invasive species *see* [invasive species](#)
- introns
  - definition [532](#)
  - genetic variability [56](#)
  - selection [220](#)
- invasive species [360](#), [414–428](#)
  - adverse effects [415](#)
  - biodiversity effects [415–416](#)
  - control of [420–425](#)
    - ballast water treatment [420](#)
    - biocide resistance [423](#)
    - biological control [419–420](#), [421](#)
    - genetic control [423–425](#)
    - immunocontraception [421–422](#), [422](#)
    - monitoring [425](#)
  - definition [532](#)
  - evolution effects [418–420](#)
    - adaptive changes [418](#)

- of native species 419–420
- genetics 418, 418
- hybridization 417
- introgression 425–427
  - alleviation of 427
  - definition 425
  - detection 426
  - transgenics 426–427
- phases 416–418
  - establishment 416
  - introduction 416
  - lag phase 417
  - population expansion/spread 417
- scale of 415
- source populations 417
- inversions
  - definition 532
  - genetic diversity 56
- Ipomopsis aggregata*
  - see also scarlet gilia
- ISIS, captive breeding records 432
- island populations
  - extinctions 3, 4, 26
    - human factors 26
  - gene flow 325, 325
  - management 355
  - population fragmentation see population fragmentation
- isolation, complete see population fragmentation
- isolation by distance 311–312, 330, 331
- isozymes, definition 533
- IUCN
  - captive breeding programs 431
  - definitions 5, 5, 6, 533
  - extinction risk categorization, population size 341
  - Red Books 6

Japanese quail (*Coturnix japonica*), inbreeding [22](#)  
Javan rhinoceros (*Rhinoceros sondaicus*), wild population sizes [344](#)  
Jersey Wildlife Preservation Trust [432](#)  
Johnson's crocodile (*Crocodylus johnstoni*) [419](#), [420](#)  
Jurassic Park scenario [448–449](#)

karyotypes [56](#)  
kidney vetch (*Anthyllis vulneria*), isolation by distance [331](#)  
Killifish (*Fundus heteroclinus*) [222](#)  
kinship ( $k_{ij}$ ) [440](#)  
    definition [533](#)  
    minimization, captive population management [443–444](#)

koala  
    gene tree [480](#)  
    population bottlenecks [479](#)  
    translocation [403](#), [404–405](#)

*Kokia cookei*

    see also Cooke's kok'io

kokolau (*Bidens amplexans*), wild population sizes [344](#)  
Komodo dragon (*Varanus komodoensis*), wild population sizes [344](#)

lag phase, invasive species [417](#)  
Lakeside daisy (*Hymenoxys acaulis* var *glabra*) [29](#)  
    outcrossing [402](#)  
    population targets [351](#)

LAMARC [495](#)

landscape genetics [331–332](#)  
    definition [331](#), [533](#)  
    population fragmentation [331](#), [332](#)

large populations [86](#)  
    genetic diversity [62](#), [184](#)  
        small populations vs. [183](#)  
    selection [478](#) see [evolution](#)  
        migration see [migration](#)  
        mutation see [mutation](#)

*Lasiiorhinus krefftii* see [northern hairy-nosed wombat](#)

Laysan finch (*Telespiza cantans*)  
  genotype frequencies [67](#), [68](#)  
  microsatellites, expected heterozygosity [72](#), [80](#), [84](#)  
*Lemniscomys barbarus*  
  see also striped grass mouse  
*Leontopithecus chrysomelas*  
  see also golden-headed lion tamarin  
*Leontopithecus rosalia* see [golden lion tamarin](#)  
leopard (*Panthera pardus*), population viability analysis [511](#)  
*Lepus americanus*  
  see also snowshoe hare  
lethal, definition [533](#)  
lethal alleles, mutation–selection balance [150](#)  
lethal equivalents (*B*)  
  definition [533](#)  
  inbreeding depression see [inbreeding depression](#)  
lethal homozygotes, inbreeding [268](#), [268–269](#)  
*Leucopsar rothschildi*  
  see also Bali starling  
*Limonium dufourii*  
  see also sea lavender  
lineage sorting  
  definition [533](#)  
  phylogenetic trees [379](#)  
linear stepping stone structure see [population fragmentation](#)  
linkage disequilibrium [83–86](#), [84](#), [85](#), [86](#)  
  definition [83](#), [533](#)  
  directional selection [217](#)  
  major histocompatibility complex polymorphism [194](#)  
  measure of [84](#), [85](#)  
listing importance, endangered species [6](#)  
loci variation, mutations [142](#)  
locus (loci), definition [44](#), [533](#)  
loggerhead turtle (*Caretta caretta*)  
  dispersal [484](#), [484](#)  
  recovered populations, sensitivity analyses [510](#)

long-finned pilot whale (*Globicephala melas*), population structure [483](#)  
long-term adaptive changes, quantitative characters [134–135](#), [135](#)  
Lord Howe Island woodhen (*Gallirallus sylvestris*)  
    captive breeding, founder numbers [435](#)  
    population size increase [396](#)  
    reintroduction [469–470](#)  
    wild population sizes [344](#)  
lower risk species, IUCN definition [5](#)  
*Lycalopex fulvipes*  
    see also Darwin's fox  
lynx, population size fluctuations [244](#)  
  
*Macrotis lagotis*  
    see also bilby  
*Macroderma gigas* [483](#)  
    see also ghost bat  
mainland–island (source–sink) structure see [population fragmentation](#)  
maintenance phase, captive breeding programs [437–444](#)  
maize (*Zea mays*)  
    directional selection [135](#)  
        vs. effective population size [346](#)  
    hybrid vigour measurement [223](#)  
    inbreeding depression [287](#), [299](#)  
major histocompatibility complex (MHC) [30](#)  
    definition [533](#)  
    diversity  
        maintenance in small populations [201](#)  
        Tasmanian devils [30](#)  
    polymorphisms [57](#)  
        HIV infection [30](#)  
        individual loci genetic management [224–225](#), [226](#)  
*Malacothamnus fasciculatus*  
    see also Santa Cruz Island bush mallow  
malaria [2](#), [195](#), [215](#)  
    avian [116](#), [417](#), [494](#)  
    falciparum [189](#)



- distribution [182](#), [189](#)
- genotype frequency and [190](#)
- resistance [59](#), [189](#), [190](#), [191](#), [217](#)
- Malurus splendens* see [splendid fairy wren](#)
- mammals
  - dispersal distances [330](#)
  - genomes [208](#)
  - molecular sexing [492](#)
  - see also *individual species*
- mammoth (*Mammuthus*), genetic resurrection [208](#), [448](#)
- management units, definition [12](#), [533](#)
- maned wolf (*Chrysocyon brachyurus*), deleterious mutations [148](#)
- Mantel tests, spatial autocorrelation [332](#)
- marine protected areas [407](#)
- MARK [495](#)
- matchstick banksia (*Banksia cuneata*), population viability analysis [513–514](#)
- maternal effects, definition [533](#)
- maternal environment, quantitative variation [97](#)
- MateRX [451](#)
- mating trials, DNA barcoding [369](#)
- Mauna Kea silversword (*Argyroxiphium sandwicense* ssp. *sandwicense*)
  - outcrossing [402](#)
  - reintroduction [470](#)
  - wild population sizes [344](#)
- Mauritius kestrel (*Falco punctatus*)
  - bottlenecks [162](#), [162–163](#), [168](#), [170](#), [171](#)
  - genetic bottleneck [73](#)
- Mauritius pink pigeon see [pink pigeon \(\*Columba mayeri\*\)](#)
- maximum avoidance of inbreeding (MAI), captive population management [442](#), [444](#)
- maximum likelihood [533](#)
- maximum parsimony methods, phylogenetic trees [377](#), [378](#)
- McDonald–Kreitman test [218](#), [218–219](#)
- mean [101](#)
- mean kinship [440](#), [440](#), [441](#)

- minimization [439–441](#)
- mean kinship breeding strategies [441–442](#)
- MEDARKS (Medical Animal Record Keeping System) [432](#)
- Medical Animal Record Keeping System (MEDARKS) [432](#)
- Megaptera novaeangliae* see [humpback whale](#)
- Melanerpes formicivorus* (acorn woodpecker) [509](#)
- melanism see [industrial melanism](#)
- Meleagris* (turkey), inbreeding depression [287](#)
- Melitaea cinxia*
  - see also [Glanville fritillary butterfly](#)
- melon fruit fly (*Bactrocera cucurbitae*) [423](#)
- meta-analyses [15](#), [30–33](#)
  - combined probability method [31](#)
  - definition [533](#)
  - effect size analysis [32](#)
  - Fisher's combined probability test [32](#)
  - publication bias [32](#)
  - vote-counting method [31](#)
- MetaMK [451](#), [471](#)
- metapopulations [312](#), [313](#), [313](#)
  - definition [533](#)
  - reproductive fitness effects [333](#), [333](#), [334](#), [334](#)
- methodology (in conservation genetics) [15](#)
- metric variation see [quantitative variation](#)
- Mexican wolf (*Canis lupus baileyi*)
  - inbreeding [173](#), [174](#)
  - reintroduction [467](#)
  - sub-species misclassification [365](#), [491](#)
- mice
  - directional selection [135](#)
    - effective population size vs. [346](#)
  - dispersal and gene flow [485](#)
  - diversifying selection [198](#)
  - gene expression studies [222](#)
  - genetic diversity, quantitative variation [60](#), [93](#)
  - genome sequencing [208](#), [214](#)

- heterozygote advantage [192](#), [195](#)
- inbreeding
  - linearity [299](#)
  - variability [295](#), [296](#)
- inbreeding depression [22](#), [23](#), [23](#), [286](#), [287](#), [293](#), [294](#)
- individual-locus genetic management [226](#)
- mutation rate [143](#)
- outbreeding depression [381](#), [383](#)
- population size, genetic diversity [239](#)
- purging [299](#)
- quantitative characters [135](#)
- reproductive fitness [341](#)
- rodenticide resistance [119](#), [423](#)
- selection
  - non-coding DNA [221](#)
  - small populations [176](#), [346](#)
  - transposable genome [215](#), [215](#)
- microarrays [51](#), [209](#), [225](#)
  - benefits/uses [211](#), [222](#)
  - definition [534](#)
  - see also* [cDNA expression microarrays](#)
- Micronesian kingfishers (*Halcyon cinnamomina cinnamomina*) [492](#)
- microRNA (miRNAs) [214](#)
  - definition [533](#)
- microsatellites
  - bottlenecks [170](#), [170](#), [479](#)
  - CA repeats [50](#), [58](#), [59](#)
  - definition [533](#)
  - genetic diversity [49](#), [50](#), [54](#), [58–59](#), [65](#)
  - pedigree reconstruction [490](#)
  - population size [478](#)
  - threatened species [61](#), [62](#)
  - variation, genetic diversity loss [237](#), [238](#)
- MIGRATE [335](#)
- migration [141](#), [152–141](#), [153](#)
  - clines *see* [clines](#)

- definition 533
- evolution 120
- gene flow 325, 326, 326
- genetic diversity 141
- gene trees 477, 477
- migration corridors 400, 401
  - advantages 400
  - definition 529
- migration–selection equilibria 155–158
  - clines 158
  - models 157
- Millennium Seed Bank 459
- Milvus milvus fasciicanda*
  - see also Cape Verde kite
- minimum habitat area 519, 519
- minimum viable population (MVP) 338, 517–520
  - definition 533
  - minimum habitat area 519, 519
  - study duration effects 518
- minisatellites 533
- MINITAB 112
- mcRNA 214
  - definition 535
- Mirounga angustirostris* see northern elephant seal
- mismatch analysis, forensic demographic history 478
- mitochondrial DNA (mtDNA)
  - allopatric speciation 374, 379
  - coalescence 477
  - definition 533
  - diversity vs. population size 240
  - genetic diversity measurement 50, 54–55
  - heterozygosity loss in small populations 241
  - restriction fragment length polymorphisms 54
  - sequencing 54
  - single strand conformational polymorphisms 54
- MLNE 257

- moa (*Diornis*), molecular sexing 492
- models/simulations 15
  - extinction due to inbreeding 27
  - gene flow management 401, 403
  - genetic drift 164, 165
  - inbreeding 20
  - inbreeding depression 296, 297
  - individual loci genetic management 226
  - population evolution 120, 121
  - small populations 165, 178, 179
- mode of inheritance, genetic diversity measurement 54
- molecular clocks 495
  - definition 534
  - forensics 494
- molecular genetic variation, quantitative variation vs. 110
- molecular operational taxonomic units (MOTU) 369
  - definition 534
- monard butterfly (*Danavs plexippus*) 427
- monitoring, invasive species control 425
- mongoose 421
- monomorphic 44, 534
- monophyletic 534
- monophyly, reciprocal 385
- moose (*Alces alces*) 273, 406
- morphology, species definition 363, 367
- mortality, reintroduction 463
- moving target hypothesis see rare allele advantage (frequency-dependent selection)
- MrBayes 388
- MSVAR 495
- multigene families, copy number variation 215
- multigeneration effects see population fragmentation
- multilocus data, breeding systems 488
- multilocus microsatellites, gene flow 328
- multiple gene copies, population genomics 212
- multiple loci, inbreeding depression 298

*Muntiacus muntjak* see [Indian muntjac](#)  
*Muntiacus reevesi* see [Chinese muntjac](#)  
*Mus musculus* see [mice](#)  
mustard plant (*Brassica* sp) [426](#)  
*Mustela nigripes* see [black-footed ferret](#)  
mutation(s) [141](#), [142–145](#)  
    advantageous [146](#)  
    chromosomal [142](#)  
    definition [142](#), [534](#)  
    deleterious see [deleterious mutations](#)  
    environmental agents [142](#)  
    evolution [120](#)  
    fate of [183–184](#)  
    genetically viable populations [342](#)  
    genetic diversity [141](#)  
    genome targets [214](#)  
    loci variation [142](#)  
    neutral [142](#), [146](#)  
    phenotype effects [183](#)  
    selective value [146](#)  
    silent [142](#)  
    single-locus [143](#)  
    sites [221–222](#)  
    variable effects [183](#)  
mutational meltdown [348](#), [534](#)  
mutation–drift equilibrium, single-locus genetic diversity [347](#)  
mutation load [141](#), [148](#), [178](#)  
    allele frequencies [148](#)  
    definition [60](#), [534](#)  
    deleterious alleles [147](#), [148](#)  
mutation rates [142](#)  
    estimation, mutation–selection balance [151](#), [151](#)  
    population genomics [212](#)  
    quantitative characteristics [142](#)  
    recessive lethal mutations [146](#)  
mutation–selection equilibrium [146–152](#)

- definition 147, 534
- deleterious mutation accumulation 146, 147
- equilibrium frequencies 149, 150, 151
- fitness 152, 162–163
- genetic diversity maintenance 146
- inbreeding 279, 280
- lethal alleles 150
- mutation rate estimation 151, 151
- polyploids 151
- recessive alleles 150
- sex-linked loci 150
- small populations 177
  - genetic diversity maintenance 199
- tetraploids 150, 150
- see also mutation load

myxomatosis 421

myxoma virus

- Australia, introduction to 421
- resistance evolution 118, 118

*Naja nigricollis*  
see also African black-necked cobra

naked mole rat (*Heterocephalus glaber*), inbreeding depression 302

*Nasella pulchra*, local condition adaptation 462

natural selection

- definition 534
- large populations 478

ncRNA 207, 212, 213, 214, 535

Neanderthals 208

near neutral theory of molecular evolution 188

- genome complexity 216
- mild deleterious mutations 188

NeEstimator 257

Nei's genetic distance ( $D_N$ )

- allopatric speciation 376–377
- definition 534

- Nei's genetic similarity ( $I_N$ ) 534
- nematodes 146, 210, 221, 295, 349
  - gastrointestinal 195, 295
- nene (*Branta sandvicensis*)
  - bottlenecks 170
  - population size increase 396
- $N_e/N$  ratios see effective population size ( $N_e$ )
- neutral loci, genetic diversity loss 237, 238
- neutral mutations 142, 146, 147, 183
  - definition 534
  - genetic drift 184
  - under random genetic drift see genetic diversity maintenance
  - removal 184
- neutral theory of molecular evolution 185
  - coalescence 255
  - genetic diversity see genetic diversity
- NEWHYBRID 429
- NEWPAT 495
- Ninox novaeseelandiae* see Norfolk Island boobook owl
- non-additive genetic variation, bottlenecks 173
- non-ancestral derived alleles, directional selection 217
- non-coding DNA selection, evolution 220–221
- non-coding RNA (ncRNA) genes 207, 212, 213, 214, 535
- non-idealized populations, gene flow 327
- non-obtrusive sampling 12
- non-synonymous substitutions
  - definition 534
  - directional selection 216
  - major histocompatibility complex polymorphism 193
- non-threatened species, captive population management 444
- non-translated DNA, neutral mutations under random genetic drift 186
- Norfolk Island boobook owl (*Ninox novaeseelandiae*)
  - backcrossing 277, 278, 278–279
  - molecular sexing 492
  - phylogenetic trees 377, 378



normal distribution, definition [534](#)  
 North American pika (*Ochotona princeps*), dispersal [484](#)  
 North American puma, taxonomic uncertainty [364](#)  
 Northern Atlantic right whale (*Eubalaena glacialis*), wild population sizes [344](#)  
 northern elephant seal (*Mirounga angustirostris*)  
     bottlenecks [162](#), [168](#), [169](#), [480](#), [480–481](#)  
     effective population size measurement [244](#), [245](#)  
     genetic viability [338](#), [339](#)  
     inbreeding [272](#)  
     inbreeding depression [293](#)  
     population size increase [394–396](#)  
     unequal sex-ratio [249](#), [250](#)  
 northern hairy-nosed wombat (*Lasiorhinus krefftii*)  
     effective population size measurement [253](#)  
     forensic biology [474](#), [475](#)  
     genetic diversity [240](#)  
     non-obtrusive sampling [12](#)  
     wild population sizes [344](#)  
 northern quoll (*Dasygurus hallucatus*) [419](#), [420](#)  
 northern spotted owl (*Strix occidentalis caurina*), isolation by distance [331](#)  
 nuclear DNA, genetic diversity [56–57](#)  
 nucleotide diversity ( $\pi$ )  
     definition [534](#)  
     expected heterozygosity [76](#)  
 null alleles [490](#), [534](#)  
*Numenius madagascariensis*  
     see also curlew  
 nyala (*Tragelaphus angasii*), heterozygosity vs. generation time [352](#)  
 observed heterozygosity ( $H_o$ ) [68](#), [69](#)  
*Ochotona princeps*  
     see also North American pika  
*Odocoileus virginianus*  
     see also white-tailed deer

okapi (*Okapia johnstoni*), inbreeding coefficient vs. survival [306](#)

old-field mice, inbreeding depression [295](#)

*Onchyophora*

*see also* velvet worm

*Oncorhynchus tshawytscha* *see* [Chinook salmon](#)

opossum (*Didelphis virginiana*) [210](#)

orangutan (*Pongo*)

mean population size [512](#)

population viability analysis [512](#)

sub-species [369](#), [374–375](#)

misclassification [365](#), [374–375](#)

molecular biology [374](#), [374–375](#)

*see also individual sub-species*

oriental fruit fly (*Bactrocera dorsalis*) [423](#)

*Oryctolagus cuniculus*

*see also* rabbit

*Oryx dammah*

*see also* scimitar-horned oryx

*Oryx leucoryx* *see* [Arabian oryx](#)

outbred populations, directional selection [134](#)

outbreeding, [9](#), [23](#), [28](#), [367](#), [458](#), [488](#), [488](#)

computer projections on inbreeding and [27](#)

definition [536](#)

depression *see below*

DNA finger prints [52](#)

gene flow [328](#)

genetic diversity [41](#), [42](#), [56](#), [58](#), [141](#)

Hardy–Weinberg equilibrium [71](#)

inbreeding concerns vs [262](#), [265](#), [268](#), [291](#), [292](#), [294](#)

inbreeding depression

*see also* inbreeding depression

laboratory animals [15](#)

mutation load/effects [147](#), [152](#), [348](#), [349](#)

mutation–selection balance [152](#), [279](#)

protein variation [58](#)

quantitative variation [60](#), [96](#), [97](#), [106](#)

- reintroduction [464](#)
- selfing vs [507](#)
- small founder numbers [436](#)
- outbreeding depression [9](#), [307](#), [381–384](#)
  - coadapted gene complexes [383](#)
  - definition [91](#), [363](#), [534](#)
  - dispersal characteristics [382](#)
  - environment effects [381](#)
  - erroneous taxonomy [382](#)
  - extent [381–382](#)
  - genetic basis [383–384](#)
- outcrossing
  - definition [534](#)
  - small population genetic rescue [397–399](#), [398](#)
- overdominance see [heterozygote advantage \(overdominance\)](#)
- overdominant loci, genotypic variables [98](#), [98](#)
- overlapping generations, effective population size measurement [251](#)
- Ovis canadensis* see [bighorn sheep](#)
  
- Pacific yew (*Taxus brevifolia*), population fragmentation [322](#), [324](#)
- Palo Verdes blue butterfly (*Glaucopsyche lygdamus palosverdesensis*),  
wild population sizes [344](#)
- PAML [3–5](#), [228](#)
- panmictic [535](#)
- Pan paniscus*
  - see also bonobo
- Panthera leo*
  - see also African lion
- Panthera leo persica* see [Asiatic lion](#)
- Panthera pardus*
  - see also leopard
- Panthera tigris*
  - see also tiger
- Panthera tigris altaica* see [Siberian tiger](#)
- Panthera tigris sumatrae*
  - see also Sumatran tiger

*Pan troglodytes* see [chimpanzee](#)

parentage see [forensics](#)

partial dominance

definition [535](#)

selection [127](#)

PARTITION [335](#)

partitioning

environmental variation [91–92](#), [96](#), [96–97](#)

quantitative variation [97–99](#)

single loci variation [98](#), [98](#), [98](#)

*Partula* see [Tahitian land snail](#)

*Parus major*

see also [tit](#)

*Patagioenas inornata*

see also [plain pigeon](#)

pathogens

as biological control [421](#)

continuing evolution [116](#)

newly identified [493](#)

resistance to, major histocompatibility complex polymorphism [194](#)

spread of [116](#)

PAUP [389](#)

*Pedicularis furbishiae*

see also [Furbish's lousewort](#)

PEDIGREE [496](#)

pedigree(s)

captive population management [443](#)

definition [535](#)

inbreeding see [inbreeding](#)

reconstruction

forensics [490](#)

microsatellites [490](#)

*Perameles gunni* see [Eastern barred bandicoot](#)

Père David deer (*Elaphurus davidianus*), bottlenecks [169](#)

peregrine falcon (*Falco peregrinus*), population targets [351](#)

peripheral characters

- definition [535](#)
- selection response [105](#), [106](#)
- Peromyscus leucopus*
  - see also white-footed mouse
- Petauroides volans*
  - see also greater glider
- Petrogale lateralis* see [black-footed rock wallabies](#)
- Petroica traversi* see [Chatham Island black robin](#)
- phenotype resemblance, quantitative variation [96](#), [100–102](#)
- phenotypic plasticity [535](#)
- phenotypic variance ( $V_p$ ) [96](#)
- phenylketonuria [148](#)
- phlox (*Phlox cuspidata*) [78](#), [79](#)
- pine tree (*Pinus* sp) [91](#), [311](#)
  - invasive in South Africa [415](#)
  - Monterey pine [329](#), [330](#)
  - Scots pine [295](#)
  - Wollemi see [Wollemi pine](#)
- Phoenicopterus ruber*
  - see also Caribbean flamingo
- PHVA see [population and habitat viability assessments \(PHVA\)](#)
- PHYLIP [389](#)
- phylogenetic species concept [367](#)
- phylogenetic trees [377–379](#)
  - data [377](#)
  - definition [535](#)
  - lineage sorting [379](#)
  - maximum parsimony methods [377](#), [378](#)
  - polymorphisms [379](#), [387](#)
  - statistics [377](#)
- phylogeography
  - definition [535](#)
  - forensics [485–486](#)
- physical factors, speciation [370](#)
- physiological adaptation [116](#)

*Picoides borealis* see [red-cockaded woodpecker](#)

pigs, inbreeding depression [287](#)

pink pigeon (*Columba mayeri*)

- bottlenecks [170](#)

- captive breeding, founder numbers [435](#)

- heterozygosity vs. generation time [352](#)

- reproductive success/family size [246](#)

- wild population sizes [344](#)

plain pigeon (*Patagioenas inornata*), captive breeding [318](#)

plants

- adaptive evolution [117](#)

- cloning [448](#)

- DNA barcoding [363](#)

- ex situ* conservation [446](#)

  - generation minimization [459](#)

- genetically viable populations [341](#)

- genetic diversity [62](#)

- heavy-metal tolerance [42](#)

- inbreeding depression [23](#)

- polyploidy [371](#)

- see also *individual species*

platypus (*Ornithorhynchus anatinus*) [210](#)

ploidy, inbreeding depression [299](#)

PM2000 [357](#), [452](#)

pocket gopher (*Thomomys*) [11](#), [374](#), [374](#)

Poisson distribution

- definition [535](#)

- idealized populations [175](#)

polyandry [249](#)

- definition [535](#)

polygamy [535](#)

polygene, definition [535](#)

polygenic characters see [quantitative variation](#)

polygyny [249](#)

- definition [535](#)

polymerase chain reaction (PCR)

- definition [535](#)
- genetic diversity measurement [48](#), [49](#)
- polymorphic [535](#)
- polymorphic genetic markers, population structure [482](#)
- polymorphisms
  - definition [44](#)
  - genetic diversity [43](#)
  - major histocompatibility complex [57](#)
  - phylogenetic trees [379](#), [387](#)
  - plant self-incompatibility locus [57](#)
  - population genomics [209](#)
- polyphyletic [535](#)
- polyploid loci
  - definition [535](#)
  - genetic diversity loss [241](#), [242](#)
- polyploids
  - Hardy–Weinberg equilibrium [82–83](#)
  - inbreeding [281–282](#)
  - mutation–selection balance [151](#)
  - plants [371](#)
  - speciation [371](#)
  - wild population management [412](#)
- Pongo* *see* [orangutan](#)
- Pongo abelii*
  - see also* Sumatran orangutan
- Pongo pygmaeus* *see* [Bornean orangutan](#)
- POPGENE [87](#)
- poplar (*Populus* sp) [210](#)
- population(s)
  - definition [313](#), [314](#)
  - evolution [119–121](#)
    - controlling factors [141](#)
    - models [120](#), [120](#), [121](#)
    - see also specific controlling factors*
  - expansion/spread, invasive species [417](#)
  - fluctuation, genetic diversity loss [238](#)

- genetically viable *see* [genetically viable populations](#)
- genetic constituents [39](#)
- growth rates, inbreeding depression [291](#)
- idealized *see* [idealized populations](#)
- inbreeding depression *see* [inbreeding depression](#)
- large *see* [large populations](#)
- reintroduction [464](#)
- small *see* [small populations](#)
- structure
  - forensics [484](#)
  - haplotype networks [482](#), [483](#)
  - polymorphic genetic markers [482](#)
- population and habitat viability assessments (PHVA) [433](#)
  - definition [535](#)
  - population viability analysis [515](#)
- population fragmentation [309](#), [310](#), [317](#)
  - captive breeding programs *see* [captive population management](#)
  - complete isolation [314–321](#)
    - initial generation effects [316–317](#), [317](#), [320](#)
    - see also specific types*
  - Drosophila* [318](#)
  - gene flow *see* [gene flow](#)
  - genetic barriers [310](#)
  - genotype frequencies [320](#)
  - habitat fragmentation [230](#)
  - idealized populations [316](#), [316](#)
  - island structure [312](#), [313](#), [315](#), [315](#)
    - gene flow [325](#), [325](#)
    - reproductive fitness effects [333](#)
  - landscape genetics [331](#), [332](#)
  - linear stepping stone structure [312](#), [313](#)
    - reproductive fitness effects [333–334](#)
  - mainland–island (source–sink) structure [312](#), [313](#)
    - reproductive fitness effects [333](#), [334](#)
  - measurement [321–325](#)
  - metapopulations *see* [metapopulations](#)



- models [312](#), [313](#)
- multigeneration effects [317](#), [321](#)
  - allele frequencies [318](#), [319](#)
  - $F$  statistics [323](#)
  - genotype frequencies [320](#)
  - habitat fragmentation [311–312](#), [315](#)
  - heterozygosity [319](#)
  - heterozygosity reduction [320](#)
  - Wahlund effect [319](#), [320](#)
- population size reduction [230](#)
- reproductive fitness effects [334](#)
- small populations [324](#), [324](#)
- structure types [312–313](#)
  - see also specific types*
- two-dimensional stepping stone structure [312](#), [313](#)
  - reproductive fitness effects [333](#)
- population genetics [204](#)
- population genomics [207–228](#)
  - benefits from [211–212](#)
  - definition [535](#)
  - deleterious allele detection [212](#)
  - evolution *see* [evolution](#)
  - gene expression studies [222–223](#)
  - individual loci genetic management *see* [genetic management](#)
  - polymorphic sequence identification [209](#)
  - see also* [genome\(s\)](#); [genome sequencing](#)
- population size
  - captive population management [438](#)
  - definition [242](#)
  - fluctuations, effective population size measurement [244](#), [244](#), [245](#)
  - forensics [478](#)
  - genetic diversity vs. *See* [genetic diversity](#)
  - habitat fragmentation [310](#)
  - harmonic mean [244](#)
  - inbreeding vs. [282](#)
  - microsatellites [478](#)

- reduction in 229
  - affecting factors 229
  - genetically viable populations 230
  - genetic diversity loss 229–230
  - inbreeding 230
    - see also inbreeding
  - population fragmentation 230
    - see also population fragmentation
  - sustained restrictions see genetic diversity loss
  - threatened species 14
- population viability analysis (PVA) 15, 464
  - accuracy 515–516, 515
  - case studies 510–514
  - data restrictions 516
  - definition 500, 500, 519, 535
  - development 501
  - extinction see extinction
  - genetics 505–506
    - extinction risk 506
    - family size 506–507
    - inbreeding depression 505, 506
  - limitations 516–517
  - mechanism 501, 501
  - recovered populations 509–514
    - sensitivity analyses 510
  - software 504
    - VORTEX 501, 502
  - stochastic *r*-models 504, 517
  - utility 514–516
    - process vs. 514–515
  - viable population definition 517
- Potentilla glandulosa*
  - see also sticky cinquefoil
- potoroo, speciation 372, 373
- potosi pupfish (*Cyprinodon alvarezi*) 430
- predation, rare allele advantage 192

preferential breeding control, captive population management [459](#)  
prickly pear cactus (*Opuntia* sp) [414](#), [415](#), [421](#)  
primers [536](#)  
probes [536](#)  
*Procyon lotor*  
    *see also* racoon  
progeny numbers, inbreeding depression [290](#)  
proportion of loci polymorphic (*P*) [44](#)  
protein(s)  
    genetic diversity [45](#), [59](#), [59](#)  
    genetic diversity measurement [46–48](#)  
    separation, gel electrophoresis [46](#)  
protein coding loci [213](#)  
    genomes [212](#)  
Przewalski's horse (*Equus ferus przewalskii*)  
    bottlenecks [169](#)  
    captive breeding, founder numbers [435](#)  
    deleterious mutations [148](#)  
    inbreeding [261](#), [261–262](#), [269](#)  
        pedigrees [274](#)  
    reintroduction [467–468](#)  
    reproductive success/family size [246](#)  
pseudogenes [213](#)  
    definition [536](#)  
*Pseudoryx nghetinhensis* (saola) [375](#)  
pufferfish [210](#)  
publication bias, meta-analysis [32](#)  
Puerto Rican parrot (*Amazona vittata*)  
    bottlenecks [170](#)  
    captive breeding, founder numbers [318](#)  
    population viability analysis [509](#)  
    wild population sizes [344](#)  
*Puma concolor* *see* [Florida panther](#)  
purging [9](#), [299–303](#)  
    definition [279](#), [536](#)  
    deleterious mutations [300](#)

- purifying selection 221
  - definition 536
  - non-coding DNA selection 221
- puritan tiger beetle (*Cicindela puritana*), taxonomy 387
- pygmy hippopotamus (*Hexaprotodon liberiensis*), inbreeding 262, 263
- Pyrenean brown bear (*Ursus arctos*), molecular sexing 492
- qualitative characters
  - genetic diversity measurement 55
  - quantitative vs. 93
- quantitative characters 92–94
  - clines 156
  - definition 536
  - directional selection 133, 133, 134
  - disruptive selection 133
  - genotype vs. phenotype 92, 93
  - heritability *see* heritability
  - qualitative vs. 93
  - selection 133–136, 133
    - directional selection 134
    - disruptive selection 136
    - long-term adaptive changes 134–135, 135
    - stabilizing selection 135–136, 136
    - see also specific types*
  - stabilizing selection 133
- quantitative trait loci (QTLs) 94
  - definition 536
- quantitative variation 90
  - basis of 94
  - bottlenecks 172–173, 173
  - definition 536
  - detection methods 94–96
  - dominance variance *see* dominance variance ( $V_D$ )
  - environmental variation 93
  - genetic diversity 60–61
  - genotype/environment interactions 110–111

importance of [91](#), [91–92](#)  
interaction variance *see* [interaction variance \( \$V\_I\$ \)](#)  
measurement over time [109–110](#)  
molecular genetic variation vs. [110](#)  
partitioning [97–99](#)  
    *see also* [additive genetic variance \( \$V\_A\$ \)](#)  
phenotype resemblance [96](#), [100–102](#)

rabbits (*Oryctolagus cuniculus*), biological control in Australia [421](#)  
raccoon (*Procyon lotor*), deleterious mutations [148](#)  
rainbow trout (*Oncorhynchus myteiss*) [287](#), [426](#)  
RAMAS [521](#)

*Rana catesbeiana* (bullfrog) [352](#)

random genetic drift [164](#)

    definition [536](#)

    neutral mutations *see* [genetic diversity maintenance](#)

randomly amplified polymorphic DNA (RAPD)

    definition [536](#)

    genetic diversity measurement [51](#), [54](#)

random mating [536](#)

RAPD *see* [randomly amplified polymorphic DNA \(RAPD\)](#)

Raphanus [418](#)

rare allele advantage (frequency-dependent selection) [116](#), [184](#), [184](#), [536](#)

    balancing selection [192–195](#)

    definition [207–228](#), [531](#)

    major histocompatibility complex polymorphism [192](#), [193–195](#)

    resource-dependency [192](#)

    self-incompatibility [195](#), [195–196](#)

rarefaction analysis, DNA-based censuring [478](#)

rat (*Rattus* sp) [134](#)

    biological control [421](#)

    brown rat [425](#)

    bush rat [201](#)

    eradication [425](#)

    fecundity in captivity [458](#)

- genetic impact of fragmentation [329](#)
- non-coding DNA [221](#)
- rodenticide resistance [119](#), [192](#)
- rattlesnakes, reproductive fitness [93](#)
- Rattus norvegicus*
  - see also brown rats
- real population size, effective population size vs. [176](#)
- recessive alleles
  - allele frequency [76](#)
  - mutation–selection balance [150](#)
- recessive homozygotes, inbreeding [267](#)
- recessive lethals
  - Drosophila* [124](#), [124](#)
  - mutation rates [146](#)
  - selection [122–124](#), [124](#)
- reciprocal monophyly [385](#)
- recorded extinctions [3](#), [3–4](#)
- recurrence relationships, inbreeding [277](#)
- red clover (*Trifolium pratense*) [463](#)
- red-cockaded woodpecker (*Picoides borealis*)
  - isolation by distance [311–312](#), [331](#)
  - population fragmentation [310](#), [311–312](#), [317](#), [318](#)
  - population targets [351](#)
  - wild population sizes [344](#)
- red-crowned crane (*Grus japonensis*), reproductive success/family size [246](#)
- red flour beetles (*Tribolium castaneum*), [165](#), [199](#), [292](#), [346](#)
  - directional selection vs. effective population size [346](#)
  - genetic drift models [164](#), [165](#)
  - small population selection models [165](#), [176](#)
- Red Queen hypothesis see rare allele advantage (frequency-dependent selection)
- red ruffed lemur (*Varecia rubra*)
  - bottlenecks [170](#)
  - captive breeding, founder numbers [435](#)
  - deleterious mutations [148](#)

- red wolf (*Canis lupus rufus*), misclassification 365, 366
- regeneration times, genetic diversity 144–145, 146
- regression 102
- regularly inbred species 296
- reintroduction 360, 453–456
  - captivity-induced genetic changes 456–457
    - see also* captive breeding programs
  - case studies 466–470
    - see also individual species*
  - definition 536
  - examples 454–456
  - forensics 475, 486
  - genetic management 461–464
  - genotype choice 462–463
    - genetic diversity 462, 463
  - individual choice 463–464
  - mortality 463
  - population choice 486
  - population numbers 464
  - site choice 461–462, 486
  - site identification 12
  - successes 464–465, 465
    - see also individual species*
    - see also* supportive breeding
- relative fitness 122, 123, 124, 126, 537
  - definition 538, 561
  - degrees of dominance, models 127, 127, 128
  - heterozygote advantage 190, 196
  - inbreeding depression 294, 294, 300
  - mutations 147
  - peppered moth 131
- remnant populations, management 355
- reproductive fitness
  - balancing selection 198–199
  - captive population management 351, 456, 457
  - copy number variation 215

- definition [530](#), [536](#)
- directional selection [134](#)
- evolution [345](#), [346–347](#)
- genetically viable populations [340–341](#), [343](#)
- genetic diversity vs. [42–43](#), [232–234](#), [233](#)
- heterozygosity vs. [232](#), [234](#)
- inbreeding depression [286–287](#), [293](#)
- inbreeding effects [15](#), [262](#)
- island population structure [333](#)
- linear stepping-stone population structure [333–334](#)
- mainland–island (source–sink) population structure [333](#), [334](#)
- metapopulations [333](#), [333](#), [334](#), [334](#)
- mutations [142](#)
- mutation–selection balance [152](#), [162–163](#)
- population fragmentation [334](#)
- short-term losses [232](#)
- two-dimensional stepping-stone population structure [333](#)
- wild population management [394](#)
- reproductive isolation, population genomics [212](#)
- reproductive population size [174](#)
- reproductive technology [447–449](#)
  - artificial insemination [447](#)
- restriction enzymes [536](#)
- restriction fragment length polymorphisms (RFLPs)
  - definition [536](#)
  - genetic diversity measurement [52–53](#)
  - mitochondrial DNA [54](#)
- retinoblastoma, frequency [148](#)
- RFLPs see [restriction fragment length polymorphisms \(RFLPs\)](#)
- Rhesus monkey (*Macaca mulatta*) [210](#)
- Rhinoceros sondaicus*
  - see also Javan rhinoceros
- Rhinoceros unicornis*
  - see also Indian rhinoceros
- rice [210](#)
- rinderpest-virus [116](#)



RNAi [214](#), [536](#)  
 rodenticide [119](#), [423](#)  
 Rodriguez giant tortoise (*Geochelone vosmaeri* and *Geochelone peltastes*) [475](#)  
 rose clover (*Trifolium hirtum*) [463](#)  
 rose pink plant, inbreeding depression [294](#)  
 round-leaf honeysuckle (*Lambertia orbifolia*) [489](#)  
 Royal Botanic Gardens (Kew) [431](#)  
 rubber vine (*Cryptostegia grandiflora*) [415](#)  
*Rutidosia leptorrhynchoides* see [grassland daisy](#)  
  
*Saguinus oedipus*  
     see also cotton topped tamarins  
 salvinia [421](#)  
 sampling  
     allele frequency models [166](#), [166](#)  
     cumulative effects [165](#), [167](#)  
     individual loci genetic management [223](#)  
     non-obtrusive [12](#)  
     plant *ex situ* conservation [446](#)  
 Santa Cruz Island bush mallow (*Malacothamnus fasciculatus*) [487](#), [487](#)  
 saola (Vu Quang bovid) (*Pseudoryx nghetinhensis*) [375](#)  
*Sarcophilus harrisii* see [Tasmanian devil](#)  
 SAS/STAT [112](#)  
*Scabiosa columbaria*  
     see also scabious plant  
 scarlet gilia (*Ipomopsis aggregata*), inbreeding depression alleviation [398](#)  
 scimitar-horned oryx (*Oryx dammah*), reproductive success/family size [246](#)  
 screwworm (*Cochliomyia* sp) [423](#), [458](#)  
 sea lavender (*Limonium dufourii*) [407](#)  
 sea otter (*Enhydra lutris*), population targets [351](#)  
 secale [418](#)  
 secondary contact, forensics [481](#)  
 seed banks [446](#)

- seed production, inbreeding depression 286–287, 294
- segregation patterns, autotetraploids 281
- SELECTION 137, 180, 204
- selection 121–132
  - adaptive evolutionary change 124–125
  - additive dominance 127
  - balancing see balancing selection
  - complete dominance 127
  - directional see directional selection
  - disruptive see disruptive selection
  - dominance 127, 127, 129, 130
  - frequency-dependent see rare allele advantage (frequency-dependent selection)
  - generation number for allele frequency change 130–131
  - inbreeding depression effects see inbreeding depression
  - intensity variation, genetic diversity maintenance 188–189, 201
  - introns 220, 220
  - mutation balance see mutation–selection equilibrium
  - partial dominance 127
  - population genomics 211
  - purifying see purifying selection
  - quantitative characters see quantitative characters
  - rare allele advantage see rare allele advantage (frequency-dependent selection)
  - recessive lethals 122–124, 122, 124
  - response prediction 104–105
  - small populations 165, 176, 199, 199–200, 200, 201
  - stabilizing see stabilizing selection
- selection coefficients ( $s$ ) 131, 131–132
  - definition 536
- selection differential ( $S$ ) 104
  - definition 537
- selective harvesting 406
  - reduction in 407
- selectively neutral 537
- selective sweep 217, 217

definition 537  
     gene trees 477  
 self-fertilization  
     autotetraploids 281  
     definition 537  
     forensics 487, 488  
     genetic diversity 408  
     Hardy–Weinberg deviation 78, 78  
     as inbreeding 263, 264, 276, 276, 277, 277  
     inbreeding coefficient 263  
     inbreeding depression 296  
     recurrence relationships 277  
     wild population management 408–409  
 self-incompatibility  
     allele loss vs. fitness 233, 233  
     definition 537  
     diversity maintenance in small populations, selection vs. drift 201,  
 201  
     loss, genetic diversity 29  
     polymorphisms 57  
     rare allele advantage 195, 195–196  
     reversion to 409  
     wild population management 409  
 sensitivity analyses  
     definition 537  
     population viability analysis 510  
*Sequoia sempervirens*  
     see also California redwood tree  
 sexing  
     birds 13  
     forensics 491–493  
 sex-linked 6-phosphogluconate dehydrogenase, *Heliconius* 81  
 sex-linked loci  
     definition 537  
     dominance 128, 129  
     equilibrium frequencies 149

- genetic diversity loss [241](#)
- Hardy–Weinberg equilibrium [72](#), [79](#), [80–82](#)
- heterozygosity loss in small populations [241](#)
- mutation–selection balance [150](#)
- Seychelles warbler (*Acrocephalus sechellensis*)
  - environmental variation [93](#)
  - inbreeding depression [289](#)
  - population size increase [396](#)
  - wild population sizes [344](#)
- sheep (*Ovis*), inbreeding depression [286](#)
- shotgun sequencing [208](#)
- Siberian tiger (*Panthera tigris altaica*)
  - bottlenecks [170](#)
  - captive breeding, founder numbers [435](#)
  - heterozygosity vs. generation time [352](#)
- sibling species [367](#)
  - definition [537](#)
- sickle-cell anaemia, **182**
  - heterozygote advantage [189](#), [189–190](#)
- silent mutations [142](#)
- silent substitutions
  - definition [537](#)
  - DNA variation [56](#)
- single large or several small (SLOSS) [537](#)
- single loci variation [66–81](#)
  - expected heterozygosity *see* [expected heterozygosity \( \$H\_e\$ \)](#)
  - genetically viable populations [347](#)
  - Hardy–Weinberg equilibrium *see* [Hardy–Weinberg equilibrium](#)
  - partitioning model [98](#), [98](#), [98](#)
- single-locus genetic disease [227](#)
- single-locus mutations [143](#)
- single-nucleotide polymorphisms (SNPs) [56](#)
  - definition [537](#)
  - genetic diversity measurement [51](#), [54](#), [209](#), [210](#)
- single pair, bottlenecks [171](#)

Single Population Analysis and Record Keeping System (SPARKS)  
432, 452

- single populations, genetic rescue 399
- single-strand conformational polymorphisms (SSCP)
  - genetic diversity measurement 53, 54
  - mitochondrial DNA 54
- siRNA, 214 537
- site choice, reintroduction 461–462
- ‘sixth extinction’ 2
- small populations 161–172
  - age composition 174
  - allele loss 30
  - bottlenecks *see* bottlenecks
  - chance effects 163, 163–173, 164
    - genetic drift 30, 164–165
  - computer simulations 165, 179
    - see also specific programs*
  - deleterious alleles 177, 177
    - variance 177
  - drift 177
  - genetic diversity 63
    - loss 231–232
      - see also* genetic diversity loss
  - genetic diversity maintenance *see* genetic diversity maintenance
  - genetic rescue, outcrossing 397–399, 398
  - heterozygote advantage 201, 202
  - importance 162–163
  - inbreeding *see* inbreeding
  - inbreeding depression 289, 290
  - mutation–selection equilibrium 177, 177
  - population fragmentation 324, 324
  - random mating *see* inbreeding
  - selection 165, 176
  - size measurement 174–176
    - effective population size 176
    - idealized population 174, 175, 175

- soapberry bug [420](#)
- snow goose (*Chen caerulescens*)
  - parentage determination [489](#)
  - population contacts [481](#), [482](#)
- snow leopard (*Uncia uncia*), captive breeding [435](#)
- snowshoe hare (*Lepus americanus*), population size fluctuations [244](#)
- smallpox [416](#)
- sodium fluoroacetate (1080) [139](#), [421](#)
  - resistance development [423](#)
- Somateria mollissima*
  - see also* eider duck
- Soulé, Michael [8](#), [9](#)
- source populations
  - invasive species [417](#)
  - translocation, genetic issues [403](#)
- source–sink [537](#)
- Southern blot [537](#)
- space constraints, genetically viable populations [338](#), [338–340](#)
- SPARKS (Single Population Analysis and Record Keeping System)  
[432](#), [452](#)
- Spartina anglica*
  - see also* common cordgrass
- spatial autocorrelation [331](#)
  - definition [537](#)
  - Mantel tests [332](#)
- speciation [370–372](#)
  - allopatric *see* [allopatric speciation](#)
  - definition [2](#), [537](#)
  - ‘instant’ speciation [371–372](#)
  - isolating factors [370–371](#)
    - environmental factors [370](#)
    - physical factors [370](#)
  - speed of [372](#)
  - sympatric *see* [sympatric speciation](#)
- species [537](#)
- species biology [13](#)

- major histocompatibility complex polymorphism 194
- species-dependent genetic diversity 63, 63
- species management units see taxonomy
- Species Survival Plans (SSPs) 432
- Speke's gazelle (*Gazella spekei*), captive breeding 435
- Sphenodon*
  - see also tuatara
- Spiranthes hongkongensis*
  - see also Hong Kong lady's tresses orchid
- splendid fairy wren (*Malurus splendens*)
  - extra-pair copulation 490
  - inbreeding depression 287
- spreading avens plant (*Geum radiatum*) 319, 336
- stabilizing selection
  - birth weight 135, 136
  - definition 537
  - quantitative characters 133, 135–136, 136
- stable equilibrium 145, 145, 538
- standard deviation 101
- starling (Sturnidae), reproductive fitness 93
- statistical power 538
- statistics, phylogenetic trees 377
- steepness, clines 156
- sterile male insect release, invasive species control 423
- sticky cinquefoil (*Potentilla glandulosa*), genotype–environment interactions 110, 111
- stochastic 538
- stochasticity
  - chance 163
  - demographic 7
  - environmental see environmental stochasticity
- stochastic *r*-models, population viability analysis 504, 517
- stress, environmental 294, 294
- Strigops habropilatus*
  - see also kakapo
- striped grass mouse (*Lemniscomys barbarus*), heterozygosity vs.

generation time [352](#)

*Strix occidentalis caurina*

see also northern spotted owl

STRUCTURE 2.2 [336](#)

studbooks

captive breeding programs [432](#)

definition [538](#)

*Styrax platanifolius* var. *texanus*

see also Texas snowbell

sub-species

definition [538](#)

rescue [485](#)

taxonomy [362–369](#), [374–375](#)

Sumatran orangutan (*Pongo abelii*), speciation [374–375](#)

Sumatran tiger (*Panthera tigris sumatrae*), reproductive success/family size [246](#)

supportive breeding [465–466](#)

definition [538](#)

fish hatcheries [465](#)

swamp pink (*Helonias bullata*) [336](#)

sweet vernal grass (*Anthoxanthum odoratum*) [466](#)

sycamore tree (*Platanus*) [371](#)

sympatric speciation [370](#), [372](#), [373](#)

allopatric speciation vs. [374](#)

synonymous mutations, neutral mutations under random genetic drift [186](#)

synonymous substitutions [538](#)

synonymous variants, evolution [221](#)

Tahitian land snail (*Partula*)

heritability estimation [100](#), [100–102](#), [103](#)

heterozygosity vs. generation time [352](#)

quantitative variation [100–102](#)

tandem repeats [538](#)

target population size, captive breeding programs [437](#)

Tasmanian devil (*Sarcophilus harrisii*)



- clonal tumours [224](#)
- individual loci genetic management [223](#), [224–225](#)
- major histocompatibility complex diversity [30](#)
- Tasmanian tiger (*Thylacinus cynocephalus*), genetic resurrection [448](#)
- taxa [538](#)
- taxa specificity, inbreeding depression [296](#)
- taxonomy [359](#), [362–387](#)
  - genetic distance [375–376](#)
    - size of [377](#)
  - importance [363–365](#)
    - hybridization problems [365](#)
  - phylogenetic trees *see* [phylogenetic trees](#)
  - resolution of [10–11](#), [11](#)
  - several species identified as single species [363](#)
  - single species split [363](#), [364–365](#), [365](#), [374–375](#)
  - species definition [366–369](#)
    - Biological Species Concept [367](#)
    - DNA sequence [367](#)
    - evolutionary arguments [367](#)
    - morphology [363](#), [367](#)
    - phylogenetic species concept [367](#)
    - see also* [DNA barcoding](#)
  - species management units [384–387](#)
    - evolutionary significant units [384–385](#)
    - exchangeability definition [385–387](#)
  - sub-species [362–369](#), [374–375](#)
  - threatened species [13](#)
- Taxus brevifolia*
  - see also* Pacific yew
- Tay–Sachs syndrome [148](#)
- Telespiza cantans* *see* [Laysan finch](#)
- telomeres [213](#)
  - definition [538](#)
  - lengths [493](#)
- tephritid flies (*Rhagoletis pomonella* complex) [420](#)
- tetraploids

definition 538  
equilibrium frequencies 150, 151  
Hardy–Weinberg equilibrium 82, 83  
heterozygosity loss in small populations 241  
inbreeding depression 299  
mutation–selection balance 150, 150  
Texas snowbell (*Styrax platanifolius* var. *texanus*), wild population sizes 344  
thalassaemia, 182  
    heterozygote advantage 192  
threatened populations  
    definition 538  
    genetically viable populations see genetically viable populations  
threatened species  
    genetic diversity loss 61, 62  
    genetic management 13–14, 14  
    genome sequencing 208  
    IUCN definition 3, 4, 5  
    microsatellites 61, 62  
    taxonomy 13  
three-spined stickleback fish (*Gasterosteus aculeatus*), speciation 370  
thylacine (*Thylacinus cynocephalus*), genetic resurrection 448, 449, 449  
tiger (*Panthera tigris*), deleterious mutations 148  
*Tigriopus californicus*  
    see also copepod  
time to extinction, population size 341  
tit (*Parus major*) 63  
tobacco, corolla length 94, 100–102  
topminnow fish, inbreeding depression 287, 288–289  
    genetic rescue 288–289, 306  
*Tragelaphus angasii*  
    see also nyala  
transcription levels  
    adaptive evolution 222  
    measurement, cDNA expression microarrays 210  
transcriptome 210, 211, 538

transfer RNA (tRNA) genes, genomes [213](#)  
transgene [538](#)  
transgenic canola [426](#)  
transgenic carp [424](#)  
transgenics, introgression [426–427](#)  
transient polymorphisms [183](#)  
    definition [538](#)  
translocation *see* [fragmented population management](#)  
transposable elements, genomes [215](#)  
transposons [142](#), [213](#)  
    definition [538](#)  
    genomes [212](#)  
    by species [215](#)  
trans-species polymorphisms [538](#)  
TreeBASE [389](#)  
*Tribolium castaneum* *see* [red flour beetle](#)  
*Trichosurus vulpecula*  
    *see also* Australian brushtail possums  
tuatara (*Sphenodon*), taxonomic uncertainty [364](#)  
Tumut fragmentation study [329–330](#)  
turkey (*Meleagris*), inbreeding depression [287](#)  
twin studies, quantitative variation detection [96](#)  
two-dimensional stepping-stone structure *see* [population fragmentation](#)  
*Tympanuchus cupido* *see* [greater prairie chicken](#)  
*Tympanuchus cupido attwateri*  
    *see also* Attwater's prairie chicken  
  
*Uncia uncia*  
    *see also* snow leopard  
unequal sex-ratio, effective population size measurement [249–250](#)  
unrelated founders, captive breeding programs [443](#)  
untranslated regions (UTRs) [208](#), [214](#), [538](#)  
*Ursus arctos* (brown bear) [148](#)  
*Ursus arctos* (Pyrenean brown bear), molecular sexing [492](#)  
US Endangered Species Act [367](#)  
UTRs (untranslated regions) [538](#)

*Varanus komodoensis* (Komodo dragon), wild population sizes [344](#)  
*Varecia rubra* see [red ruffed lemur](#)  
variance [101](#), [538](#)  
variation  
    additive see [additive variation](#)  
    environment see [environment variation](#) ( $V_E$ )  
    quantitative see [quantitative variation](#)  
VASSARSTATS [112](#)  
velvet worm (*Onychophora*), speciation [373](#)  
viable population definition, population viability analysis [517](#)  
viola [418](#)  
*Vipera berus* see [adder](#)  
VORTEX [413](#), [452](#), [471](#), [521](#)  
    extinctions [34](#)  
    population viability analysis [501](#), [502](#)  
vote-counting method, meta-analysis [31](#)  
vulnerable species [162](#)  
    definition [538](#)  
    IUCN definition [5](#), [5](#), [6](#)

Wahlund effect  
    definition [538](#)  
    population fragmentation [319](#), [320](#)  
Waldraap Ibis (*Geronticus eremita*) [491](#)  
warfarin [119](#), [192](#), [205](#), [423](#)  
    structure [423](#)  
WAZA (World Association of Zoos and Aquariums) [432](#)  
weeds, biological control [421](#)  
western clawed frog (*Xenopus tropicalis*) [210](#)  
whale meat, detection of [473–474](#)  
white-footed mouse (*Peromyscus leucopus*), heterozygosity vs. generation time [352](#)  
    white-naped crane (*Grus vipio*), heterozygosity vs. generation time [352](#)  
white sea bream fish (*Diplodus sargus*) [407](#)  
white-tailed deer (*Odocoileus virginianus*) [406](#)  
whooping crane (*Grus americana*)

- bottlenecks [170](#)
- population viability analysis [512–513](#)
- wild population sizes [344](#)
- wild oats (*Avena*), inbreeding [266](#)
- wild population(s)
  - genetic diversity loss [222–223](#), [240](#)
  - inbreeding [20](#), [23](#), [23–24](#), [24](#)
    - extinction [23](#), [23–24](#), [24](#)
  - management *see below*
  - numbers, captive breeding program initiation [435](#)
- wild population management [359](#), [391–397](#), [395](#)
  - asexual species [407–408](#)
  - fragmented populations *see* [fragmented population management](#)
  - genetic goals [349–350](#)
  - genetic issues [392–394](#)
    - diagnosis of [396](#), [397](#)
    - genetic diversity [394](#)
    - reproductive fitness [394](#)
  - genetic rescue *see* [genetic rescue](#)
  - gynodioecious plants [409](#)
  - haplo-diploid species [409–411](#)
  - haploids [412](#)
  - harvesting impacts [406](#), [406–407](#)
  - non-outbred diploid species [407–412](#)
    - see also individual species*
  - polyploid species [412](#)
  - population size increase [394–396](#)
    - decline cause removal [394](#)
  - population targets [350](#), [351](#)
  - reverse design genetic issues [405–406](#)
  - self-fertilizing species [408–409](#)
  - self-incompatible species [409](#)
- WINPOP 2.5 [137](#), [159](#), [204](#)
- wolf, 61, 173, [174](#), 364, 426, 468, [468](#)
  - Mexican *see* [Mexican wolf](#)
- Wollemi pine (*Wollemia nobilis*) [10](#)

cloning [448](#)  
genetic rescue [399](#)  
no genetic diversity [396](#), [399](#)  
population size [344](#)  
quantitative variation [91–92](#), [94](#)  
wild population sizes [344](#)  
World Association of Zoos and Aquariums (WAZA) [432](#)  
  
xeroderma pigmentosum, frequency [148](#)  
  
Y-linked alleles, heterozygosity loss in small populations [241](#)  
Y-specific markers, dispersal [484](#)  
  
*Zea mays* see [maize](#)  
zebra fish (*Danio rerio*) [210](#), [287](#), [424](#)  
zebra mussel (*Dreissena polymorpha*) [414](#), [415](#), [417](#)  
*Zieria prostrata*, reintroductions [486](#)  
ZIMS (Zoological Information Management System) [432](#), [452](#)  
Zoological Information Management System (ZIMS) [432](#), [452](#)  
ZooRisk [452](#)  
zoos, captive breeding programs [432–433](#)  
*Zosterops lateralis chlorocephalus* (Capricorn silvereye) [503](#), [504](#)